Arch Rheumatol 2017;32(2):112-117
doi: 10.5606/ArchRheumatol.2017.5931

ORIGINAL ARTICLE

# Dynamic Thiol/Disulphide Homeostasis in Patients With Fibromyalgia

Fatma FİDAN,[1] Berat Meryem ALKAN,[2] Fatma Gülçin UĞURLU,[1] Sinem BOZKURT,[2] Nebahat SEZER,[1] Cemile BİÇER,[3] Özcan EREL,[3] Özge ARDIÇOĞLU,[1] Selami AKKUŞ[1]

[1]*Department of Physical Medicine and Rehabilitation, Medical Faculty of Yıldırım Beyazıt University, Ankara, Turkey*
[2]*Department of Physical Medicine and Rehabilitation, Atatürk Training and Research Hospital, Ankara, Turkey*
[3]*Department of Biochemistry, Medical Faculty of Yıldırım Beyazıt University, Ankara, Turkey*

***ABSTRACT***

**Objectives:** This study aims to investigate dynamic thiol/disulphide homeostasis in patients with fibromyalgia syndrome (FMS).

**Patients and methods:** Fifty female patients with FMS (mean age 40.5±7.2 years; range 21 to 55 years) and 40 healthy female controls (mean age 39±9.4 years, range 22 to 55 years) were included in the study. Pain visual analog scale, tender points, Fibromyalgia Impact Questionnaire, and Beck Depression Inventory were evaluated. Age, body mass index (BMI), and symptom durations were also recorded. Native thiol, disulphide and total thiol levels were measured with a novel automated method.

**Results:** Serum disulphide levels were 14.7±3.4 µmol/L and 22.2±3.6 µmol/L in the FMS and control groups, respectively (p<0.001). Native thiol levels were 452.1±33.8 µmol/L and 433.5±37.6 µmol/L in the FMS and control groups, (p=0.015), while total thiol levels were 481.7±35.6 µmol/L and 477.5±38.9 µmol/L in the FMS and control groups, respectively (p=0.593). In the FMS group, disulphide/native thiol percent ratios and disulphide/total thiol percent ratios were statistically significantly lower and native/total thiol percent ratios were statistically significantly higher than those of the control group. There were no correlations between serum thiol/disulphide profiles and pain scores & clinical variables in patients with FMS.

**Conclusion:** Because of the decreased disulphide and increased native thiol levels, the thiol/disulphide balance has shifted to the reductive side. This metabolic disturbance may have a role in the pathogenesis of FMS.

***Keywords:*** Disulphide; fibromyalgia; thiol.

Fibromyalgia syndrome (FMS) is a chronic pain syndrome accompanied by widespread musculoskeletal pain, fatigue, sleep disturbances, functional disability, and mood disturbances.[1-3] It involves several factors, including abnormalities in the neuroendocrine and autonomic nervous systems, genetic factors, hormones, immune system, psychosocial variables, and environmental stressors.[4-6] Information on the physiopathological mechanisms are limited. There are studies indicating an imbalance between oxidants and antioxidants in patients with FMS.[7-10] The results are controversial. To the best of our knowledge, no report related to thiol/disulphide homeostasis in FMS has been published.

Thiols, also known as mercaptans, contain a sulfhydryl group (-SH) and have numerous functions, including a central role in coordinating the antioxidant defense network.[11] The plasma thiol pool is mainly formed by albumin and protein thiols and slightly formed by low molecular weight thiols.[12] Thiols can undergo oxidation reaction via oxidants and form disulphide bonds. The formed disulphide bonds can again be reduced to thiol groups; thus, dynamic thiol/disulphide homeostasis is maintained.[13,14]

***Received:*** January 28, 2016 ***Accepted:*** July 16, 2016 ***Published online:*** January 18, 2017

**Correspondence:** Fatma Fidan, MD. Yıldırım Beyazıt Üniversitesi Tıp Fakültesi Fiziksel Tıp ve Rehabilitasyon Anabilim Dalı, 06830 Bilkent, Ankara, Turkey.
Tel: +90 312 - 291 25 25 e-mail: drfatmafidan@hotmail.com

Dynamic thiol/disulphide homeostasis status has been shown to play critical roles in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and transcription factors, and cellular signalling mechanisms.[15,16] Abnormal thiol disulphide homeostasis states are involved in the pathogenesis of many kinds of diseases, including diabetes,[17] cardiovascular disease,[18] cancer,[19] chronic kidney disease,[20] and rheumatoid arthritis.[21]

One side of this double-sided balance, namely thiols, has been measurable since 1979. But now a new method, developed by Erel and Neselioglu,[25] can measure both variable levels separately and additively, so they can be evaluated both individually and holistically. Therefore, in this study, we aimed to investigate dynamic thiol/disulphide homeostasis in patients with FMS.

## PATIENTS AND METHODS

Fifty female patients (mean age 40.5±7.2 years; range 21 to 55 years) with FMS diagnosed according to the American College of Rheumatology criteria[22] were included in this study which was conducted between December 2014 and May 2015 at Medical Faculty of Yıldırım Beyazıd University Ankara Atatürk Training and Research Hospital. Exclusion criteria were as follows: diabetes mellitus, inflammatory, renal and cardiovascular diseases, pregnancy, use of corticosteroids, and malignancy history. The control group was composed of 40 healthy sedentary female volunteers (mean age 39±9.4 years; range 22 to 55 years) who had normal physical examinations and had no chronic diseases. The study protocol was approved by the Medical Faculty of Yıldırım Beyazıd University Ankara Atatürk Training and Research Hospital Ethics Committee, and all subjects were informed about the study and filled written consent forms. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Age, body mass index and demographic characteristics were recorded for all patients and controls. Musculoskeletal pain was evaluated using a 10-point graded scale; 0 indicated an absence of symptoms and 10 indicated the worst symptoms. FMS patients filled out the visual analog scale to evaluate pain intensity. Tenderness was evaluated by applying pressure over 18 specific body points, and the number of tender points was recorded.

The Fibromyalgia Impact Questionnaire (FIQ) was used to evaluate which patients were affected by FMS.[23] The FIQ is a self-administered tool comprising 10 items. Participants were also evaluated by a version of Beck Depression Inventory (BDI).[24]

Venous blood samples were collected in blood tubes. Serum samples were separated from cells by centrifugation at 1200×g for 10 minutes. Thiol/disulphide homeostasis tests were measured by a novel automated method described by Erel and Neselioglu.[25] Measurements were performed using a Cobas c501 chemical analyzer (Roche Diagnostics, Mannheim, Germany). Serum thiol/disulphide homeostasis values were presented as mmol/L. In short, disulphide bonds were first reduced to form-free functional thiol groups with sodium borohydride. Unused reductant sodium borohydride was consumed and removed with formaldehyde to prevent reduction of 5,5'-dithiobis-(2-nitrobenzoic acid), and all of the thiol groups, including reduced and native thiol groups, were determined after the reaction with 5,5'-dithiobis-(2-nitrobenzoic acid). All the chemicals were purchased from Merck Chemicals (Darmstadt, Germany) and Sigma Aldrich Chemie (Milwaukee, Wisconsin, USA). Additionally measuring native thiol and total thiol levels, disulphide levels were calculated via half of the difference between the total thiols and native thiols. After the determination of native (SH) and total thiols (SH+SS), disulphide amounts (SS), disulphide/total thiol percent ratios (SS/SH+SS), disulfide/native thiol percent ratios (SS/SH) and native thiol/total thiol percent ratios (SH/SH+SS) were calculated by multiplying with 100.

### Statistical analysis

Descriptive statistics were used to describe demographic characteristics. Comparative analyses of the parameters were performed using either the Mann-Whitney U test or student's t test depending on the levels of measurement. Spearman and Pearson correlation tests were used to determine relationship between variables. The level of significance was set at $p<0.05$.

**Table 1.** Demographic and clinical characteristics of fibromyalgia syndrome and control groups

| | Patients with FMS (n=50) | | | Healthy controls (n=40) | | | |
|---|---|---|---|---|---|---|---|
| | Mean±SD | Median | IQR | Mean±SD | Median | IQR | *p* |
| Age (year) | 40.5±7.2 | 41.5 | 12.25 | 39±9.4 | 39.5 | 18.25 | 0.472 |
| Body mass index | 27.5±4.8 | | | 26.6±4.1 | | | 0.33 |
| Disease duration (year) | 6.7±4.3 | | | | | | |
| Visual analog scale pain | 7.6±1.8 | | | | | | |
| Tender points | 14.5±1.6 | | | | | | |
| Fibromyalgia Impact Questionnaire score | 66.6±14.0 | | | | | | |
| Beck Depression Inventory score | 16.1±8.1 | | | | | | |

FMS: Fibromyalgia syndrome; SD: Standard deviation; IQR: Interquartile range.

**Table 2.** Comparison of thiol/disulphide profiles between fibromyalgia syndrome and control groups

| | Patients with FMS (n=50) | Healthy controls (n=40) | |
|---|---|---|---|
| | Mean±SD | Mean±SD | *p* |
| Native thiol (µmol/L) | 452.1±33.8 | 433.5±37.6 | 0.015* |
| Disulphide (µmol/L) | 14.7±3.4 | 22.2±3.6 | <0.001* |
| Total thiol (µmol/L) | 481.7±35.6 | 477.5±38.9 | 0.593 |
| Disulphide/native thiol (%) | 3.3±0.8 | 5.2±0.9 | <0.001* |
| Disulphide/total thiol (%) | 3.1±0.7 | 4.7±0.8 | <0.001* |
| Native thiol/total thiol (%) | 93.9±1.4 | 90.8±1.6 | <0.001* |

FMS: Fibromyalgia syndrome; SD: Standard deviation; * *p* value of <0.05 was considered statistically significant.

Statistical analyses were performed using IBM SPSS software version 20.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

There were no statistically significant differences in age and BMI between the groups (p>0.05). The disease duration, mean FIQ scores, and BDI scores were 6.7±4.3 years, 66.6±14.0, and 16.1±8.1 in FMS group, respectively. The demographic and clinical characteristics of FMS and control groups are presented in Table 1.

Serum disulphide levels were 14.7±3.4 µmol/L and 22.2±3.6 µmol/L in the FMS and control groups, respectively (p<0.001). Native thiol levels were 452.1±33.8 µmol/L and 433.5±37.6 µmol/L in the FMS and control groups, respectively (p=0.015), while total thiol levels were 481.7±35.6 µmol/L and 477.5±38.9 µmol/L in the FMS and control groups, respectively (p=0.593). Plasma native thiol levels were higher and plasma disulphide levels were lower in the FMS group compared to the control group, while total thiol levels were not significantly different between the two groups.

Disulphide/native thiol percent ratios and disulphide/total thiol percent ratios were 3.3±0.8 and 3.1±0.7, respectively, in the FMS group, while disulphide/native thiol percent ratios and disulphide/total thiol percent ratios were 5.2±0.9 and 4.7±0.8, respectively, in the control group. In patients with FMS, disulphide/native thiol and disulphide/total thiol percent ratios were statistically significantly lower and native/total

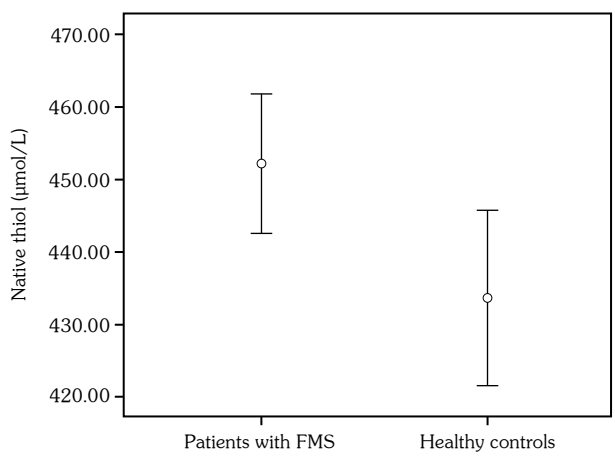


**Figure 1.** Serum native thiol levels ($x \pm \sigma_M$) in patients with fibromyalgia syndrome and healthy controls. FMS: Fibromyalgia syndrome.

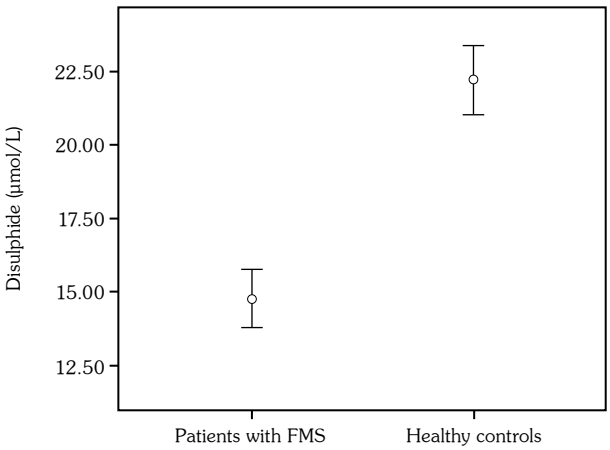


**Figure 2.** Serum disulphide levels ($x \pm \sigma_M$) on patients with fibromyalgia syndrome and healthy control groups. FMS: Fibromyalgia syndrome.

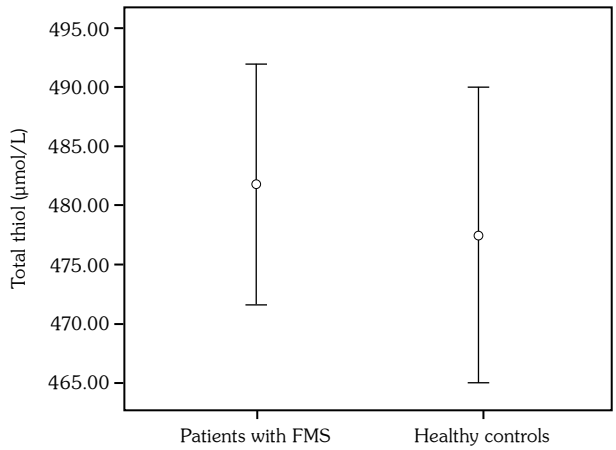


**Figure 3.** Serum total thiol levels ($x \pm \sigma_M$) in patients with fibromyalgia syndrome and healthy controls. FMS: Fibromyalgia syndrome.

thiol percent ratios were statistically significantly higher than those of the control group. The data, including the thiol/disulphide profiles of patients with FMS and controls, are given in Table 2, and Figures 1-3.

There were no correlations between serum thiol/disulphide parameters and disease duration, tender points, pain visual analog scale, and BDI and FIQ scores in patients with FMS.

## DISCUSSION

In this study, we investigated thiol/disulphide homeostasis status in patients with FMS for the first time by a recently developed method of Erel and Neselioglu.[25] Our findings showed that serum native thiol levels were higher ($p=0.015$) and serum disulphide levels were lower ($p<0.001$) in the FMS group compared to the control group ($p<0.05$), while total thiol levels were not statistically significantly different between the two groups ($p=0.593$) (Table 2). Because of the decreased disulphide and increased native thiol levels, thiol/disulphide balance has shifted to the reductive side.

The antioxidant defense system comprises enzymatic and non-enzymatic strategies. Intracellular antioxidant enzymes are mainly superoxide dismutase, catalase, and glutathione peroxidase/glutathione reductase. Non-enzymatic antioxidants are glutathione (GSH), carotenoids, tocopherols, and ascorbates.[26,27] GSH is the most important thiol-containing molecule and important intracellular antioxidant. It has a very low concentration in the extracellular milieu. It is a tripeptide made of three amino acids: cysteine, glutamic acid, and glycine. GSH protects cells from toxins, such as free radicals.[28] Sendur et al.[29] found that serum GSH and catalase levels were significantly lower in patients with FMS compared to healthy controls. Furthermore, Akbas et al.[2] showed that superoxide dismutase enzyme activity was higher in the FMS group compared to the control group, while glutathione peroxidase enzyme activity was not significantly different between the two groups. Bagis et al.[30] reported decreased serum superoxide dismutase activity in FMS. The results are controversial.

Studies of oxidant stress and antioxidant status in patients with FMS have also revealed different results; some have demonstrated a decreased total antioxidant status compared with the control groups,[8,9,31,32] whereas other studies did not show a significant difference between the two groups.[10,33] Neyal et al.[9] and Altindag and Celik[31] and Altindag et al.[32] observed decreased total antioxidant and increased total oxidant levels in FMS patients. Conversely, Bozkurt et al.[10] reported no significant differences between patients and controls' serum total antioxidant status. Chung et al.[33] concluded in their work that oxidative stress is not increased in patients with fibromyalgia. Similarly, our results also support the notion that oxidative stress is not increased in FMS patients.

In a study by Tetik et al.,[21] plasma thiol levels were shown to be decreased in rheumatoid arthritis and primary osteoarthritis patients. Similarly, Altindag et al.[32] and Sarifakioglu et al.[34] found that plasma thiol levels were significantly lower in FMS patients compared with control subjects. However, due to the current measurement failure, they were only able to measure the thiol levels of homeostasis, while they were unable to measure disulphide and total thiol levels. Hence, determination of thiol/disulphide homeostasis was not possible. But thanks to the novel method of Erel and Neselioglu,[25] we were able to measure all parameters of homeostasis, and evaluate the balance entirely and accurately. Our findings showed that plasma native thiol levels were higher and plasma disulphide levels were lower in the FMS group compared to the control group, while total thiol levels were not statistically significantly different between both groups.

In our study, we found no correlation between thiol/disulphide homeostasis results and FMS duration; number of tender points; fatigue; and visual analog scale, FIQ and BDI scores in patients with FMS. Chung et al.[33] found a correlation with oxidative stress and fatigue, but they found no correlation between oxidative stress and pain, quality of life, depression, or number of tender points. Similarly, Richard and Kahn[35] suggested that oxidative stress was associated only with fatigue but not with pain or tender points in patients with FMS.

In this study, plasma dynamic thiol/disulphide homeostasis was determined by the recently developed method by Erel and Neselioglu[25] in patients with FMS for the first time. In their preliminary studies, they showed that plasma disulphide levels were higher in patients with degenerative diseases, such as diabetes, smoking, obesity, and pneumonia, and were lower in patients with proliferative diseases, such as multiple myeloma, colon cancer, and renal cancer.[36-38] We found lower disulphide levels in patients with FMS compared with the healthy controls. However, we did not observe any difference in total thiol levels between the two groups.

The facts that the method we used in our study is new and that the literature lacks studies using this method on fibromyalgia patients were limiting in terms of comparing the results.

Although the ethiopathogenesis of FMS is still unknown, our thiol/disulphide balance results were observed to fit the pattern of proliferative disease status rather than degenerative disease status. We believe that the role of thiol/disulphide homeostasis on etiopathogenesis of FMS should be examined by future studies. Our results also support the notion that oxidative stress is not increased in FMS patients.

**Declaration of conflicting interests**

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

**Funding**

The authors received no financial support for the research and/or authorship of this article.

## REFERENCES

1. de Araújo TA, Mota MC, Crispim CA. Obesity and sleepiness in women with fibromyalgia. Rheumatol Int 2015;35:281-7.
2. Akbas A, Inanir A, Benli I, Onder Y, Aydogan L. Evaluation of some antioxidant enzyme activities (SOD and GPX) and their polymorphisms (MnSOD2 Ala9Val, GPX1 Pro198Leu) in fibromyalgia. Eur Rev Med Pharmacol Sci 2014;18:1199-203.
3. Wolfe F, Brähler E, Hinz A, Häuser W. Fibromyalgia prevalence, somatic symptom reporting, and the dimensionality of polysymptomatic distress: results from a survey of the general population. Arthritis Care Res (Hoboken) 2013;65:777-85.
4. Tutoglu A, Boyaci A, Koca I, Celen E, Korkmaz N. Quality of life, depression, and sexual dysfunction in spouses of female patients with fibromyalgia. Rheumatol Int 2014;34:1079-84.
5. Bellato E, Marini E, Castoldi F, Barbasetti N, Mattei L, Bonasia DE, et al. Fibromyalgia syndrome: etiology, pathogenesis, diagnosis, and treatment. Pain Res Treat 2012;2012:426130.
6. Bradley LA. Pathophysiology of fibromyalgia. Am J Med 2009;122:22-30.
7. Fatima G, Das SK, Mahdi AA. Oxidative stress and antioxidative parameters and metal ion content in patients with fibromyalgia syndrome: implications in the pathogenesis of the disease. Clin Exp Rheumatol 2013;31:128-33.
8. La Rubia M, Rus A, Molina F, Del Moral ML. Is fibromyalgia-related oxidative stress implicated in the decline of physical and mental health status? Clin Exp Rheumatol 2013;31:121-7.
9. Neyal M, Yimenicioglu F, Aydeniz A, Taskin A, Saglam S, Cekmen M, et al. Plasma nitrite levels,

total antioxidant status, total oxidant status, and oxidative stress index in patients with tension-type headache and fibromyalgia. Clin Neurol Neurosurg 2013;115:736-40.
10. Bozkurt M, Caglayan M, Oktayoglu P, Em S, Batmaz I, Sariyildiz MA, et al. Serum prolidase enzyme activity and oxidative status in patients with fibromyalgia. Redox Rep 2014;19:148-53.
11. Sen CK, Packer L. Thiol homeostasis and supplements in physical exercise. Am J Clin Nutr 2000;72:653-69.
12. Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. Free Radic Biol Med 2013;65:244-53.
13. Cremers CM, Jakob U. Oxidant sensing by reversible disulfide bond formation. J Biol Chem 2013;288:26489-96.
14. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. Free Radic Biol Med 2009;47:1329-38.
15. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol 2006;71:551-64.
16. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 2010;48:749-62.
17. Matteucci E, Giampietro O. Thiol signalling network with an *eye* to diabetes. Molecules 2010;15:8890-903.
18. Go YM, Jones DP. Cysteine/cystine redox signaling in cardiovascular disease. Free Radic Biol Med 2011;50:495-509.
19. Prabhu A, Sarcar B, Kahali S, Yuan Z, Johnson JJ, Adam KP, et al. Cysteine catabolism: a novel metabolic pathway contributing to glioblastoma growth. Cancer Res 2014;74:787-96.
20. Rodrigues SD, Batista GB, Ingberman M, Pecoits-Filho R, Nakao LS. Plasma cysteine/cystine reduction potential correlates with plasma creatinine levels in chronic kidney disease. Blood Purif 2012;34:231-7.
21. Tetik S, Ahmad S, Alturfan AA, Fresko I, Disbudak M, Sahin Y, et al. Determination of oxidant stress in plasma of rheumatoid arthritis and primary osteoarthritis patients. Indian J Biochem Biophys 2010;47:353-8.
22. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160-72.
23. Burckhardt CS, Clark SR, Bennett RM. The fibromyalgia impact questionnaire: development and validation. J Rheumatol 1991;18:728-33.
24. Sarmer S, Ergin S, Yavuzer G. The validity and reliability of the Turkish version of the Fibromyalgia Impact Questionnaire. Rheumatol Int 2000;20:9-12.
25. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32.
26. Chianeh YR, Prabhu K. Protein thiols as an indication of oxidative stress. Archives Medical Review Journal 2014;23:443-56.
27. Sies H. Strategies of antioxidant defense. Eur J Biochem 1993;215:213-9.
28. Schwenke DC. Antioxidants and atherogenesis. J Nutr Biochem 1998;9:424-45.
29. Sendur OF, Turan Y, Tastaban E, Yenisey C, Serter M. Serum antioxidants and nitric oxide levels in fibromyalgia: a controlled study. Rheumatol Int 2009;29:629-33.
30. Bagis S, Tamer L, Sahin G, Bilgin R, Guler H, Ercan B, et al. Free radicals and antioxidants in primary fibromyalgia: an oxidative stress disorder? Rheumatol Int 2005;25:188-90.
31. Altindag O, Celik H. Total antioxidant capacity and the severity of the pain in patients with fibromyalgia. Redox Rep 2006;11:131-5.
32. Altindag O, Gur A, Calgan N, Soran N, Celik H, Selek S. Paraoxonase and arylesterase activities in fibromyalgia. Redox Rep 2007;12:134-8.
33. Chung CP, Titova D, Oeser A, Randels M, Avalos I, Milne GL, et al. Oxidative stress in fibromyalgia and its relationship to symptoms. Clin Rheumatol 2009;28:435-8.
34. Sarıfakıoğlu B, Güzelant AY, Güzel EC, Güzel S, Kızıler AR. Effects of 12-week combined exercise therapy on oxidative stress in female fibromyalgia patients. Rheumatol Int 2014;34:1361-7.
35. Richard EH, Kahn K. Current Advances in understanding the pathophysiology of Fibromyalgia. 71st annual meeting. November 06-11, 2007; Boston, Massachusetts, USA: American College of Rheumatology; 2007.
36. Dirican N, Dirican A, Sen O, Aynali A, Atalay S, Bircan HA, et al. Thiol/disulfide homeostasis: A prognostic biomarker for patients with advanced non-small cell lung cancer? Redox Rep 2016;21:197-203.
37. Turkyilmaz E, Yildirim M, Cendek BD, Baran P, Alisik M, Dalgaci F, et al. Evaluation of oxidative stress markers and intra-extracellular antioxidant activities in patients with endometriosis. Eur J Obstet Gynecol Reprod Biol 2016;199:164-8.
38. Yuksel M, Ates I, Kaplan M, Alışık M, Erel Ö, Saygılı F, et al. The dynamic thiol/disulphide homeostasis in inflammatory bowel disease and its relation with disease activity and pathogenesis. Int J Colorectal Dis 2016;31:1229-31.