

Alteration of Thiol-Disulfide Homeostasis in Fibromyalgia Syndrome

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ABSTRACT

Background: Fibromyalgia syndrome (FMS) is an extra-articular rheumatological disease, characterized by widespread pain and somatic symptoms. The etiology has not yet been clarified. Oxidative stress may play an important role in FMS etiology. Thiol group is a very strong antioxidant. We aimed to investigate whether thiol/disulfide homeostasis in FMS is altered or not.

Material and methods: A total of 80 female FMS patients and 64 healthy female control individuals were included in this study. Thiol and disulfide values were measured by Erel's novel methods.

Results: Native thiol (330.6 ± 46.1 vs. 356.8 ± 55.5 $\mu\text{mol/L}$, $p = 0.005$) and native thiol/total thiol (89.4 ± 3.2 vs. 93.3 ± 4.0 , $p < 0.001$) levels of FMS patients were significantly lower when compared to the values of control group. However, disulfide (19.4 ± 6.3 vs. 12.2 ± 6.3 $\mu\text{mol/L}$, $p < 0.001$) levels of FMS patients were significantly higher than healthy individuals. A negative correlation was found between the native thiol/total thiol and fibromyalgia impact questionnaire (FIQ) score among the FMS patients. A positive correlation was found between disulfide values and FIQ score among the patients.

Conclusions: In FMS patients, there was a significant correlation between the decrease in the thiol levels and an increase in the disulfide levels with the FIQ scores. We determined that thiol-disulfide rate was deteriorated in FMS patients and it increases in favor of disulfide amounts.

KEYWORDS

fibromyalgia; thiol; disulfide; thiol/disulfide homeostasis; oxidative stress

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INTRODUCTION

Fibromyalgia syndrome (FMS) is an extra-articular rheumatological disease characterized by widespread pain and somatic symptoms. The etiology has not yet been clarified. It is manifested with fatigue, sleep disorders, migraine, depression, irritable bowel disease, panic disorder and decreased pain threshold (1). FMS is generally seen in the middle-aged female populations (2). Although the patients frequently experience musculoskeletal pain, the laboratory findings and the radiological examinations are normal (3). There are various studies reporting the role of genetic and biochemical markers and antibodies in the FMS pathogenesis. Polymorphisms of *5-hydroxytryptamine receptor 2A (5-HT_{2A})*, *catechol-O-methyl transferase (COMT)* gene, *serotonin transporter (SERT)* gene, *substance P* gene, and *cytokines genes (Interleukin [IL]-1 and IL-4)* are defined in FMS. All these gene polymorphisms detected in FMS are associated with monoamine metabolism and transportation. Thus, the products of these genes are severely affected by stress (4-6).

FMS has recently been associated with the deterioration of the oxidative stress and the antioxidant balance. According to the studies, it has been observed that total antioxidant capacity and compounds of the antioxidant defense system are lower in FMS patients when compared to healthy individuals (7). It has also been reported that lipid peroxidation and the oxidative stress increase in FMS. Reactive oxygen specimens (ROS) production is considerably higher in FMS patients as a result of the oxidative stress compared to controls. Increased ROS and decreased coenzyme Q10 levels in FMS are the indicators of mitochondrial dysfunction (8, 9).

Thiols may react with free radicals in order to preserve the organism against tissue and cell injury which can occur due to ROS. Thiols are the compounds that contain sulfhydryl (SH) groups (10). The highest thiol amount in blood plasma exists in albumin and others proteins (11). Thiol groups form disulfide bonds as a result of being reduced by oxidant molecules (12). These structures can turn into thiols again. When the thiol and thiol/disulfide balance is deteriorated, ROS deteriorate the protein structures and cause cellular and tissue damage (13). Erel et al. developed an automated method which can separately measure thiol/disulfide group and the evaluated thiol/disulfide rates significantly change in various diseases (10-14).

In this study, the relationship between the thiol/disulfide balance and the FMS was examined. Furthermore, we also controlled whether or not the thiol/disulfide balance is deteriorated in FMS patients when compared to healthy controls.

METHODS

This cross-sectional study was conducted in our Rheumatology, Physical Medicine and Rehabilitation clinics between January 2016 and April 2016. Eighty female patients who had complaints at least for the last three months and 64 healthy female controls were included in the study. The control group was selected among the voluntary

individuals. Patient and control groups were compatible interns of age and body mass index (BMI). Patients were selected according to the ACR 2010 diagnosis criteria (15). Disease activities of FMS patients were evaluated by using Fibromyalgia Impact Questionnaire (FIQ) scores and the sleep quality of patients was assessed by using the Pittsburg sleep quality scale (PSQS). Patients and controls with concomitant systemic situations and additional properties such as vegetarian or vegan, smoking, alcohol use, pregnancy, and breastfeeding were excluded from the study. Furthermore, patients with comorbidities such as hypertension, cardiovascular disease, acute or chronic kidney disease, infections, chronic obstructive pulmonary disease, and malignancies were not included in the study. In addition, individuals who were on any type of medication (including contraceptives) or antioxidants were not included in the control group. Patients who were on drugs other than the ones used for the FMS treatment were not included in the patient group. All of the individuals were informed about the study and their written consents were obtained. The local ethics committee approval was obtained for the study.

FIBROMYALGIA IMPACT QUESTIONNAIRE

FIQ was developed by Burckhard et al. in order to evaluate the functional status of the FMS. The Turkish reliability and validity study of the scale was conducted by Sarmer et al. The physical sufficiency was evaluated via the survey which was composed of 11 items related to the daily activities (16, 17). The questions were aiming to highlight well-being and the loss of daily work, difficulty in working, pain, fatigue, and well-being in the morning after waking up, stiffness, anxiety, and depression. High scores presented functional limitations.

PITTSBURGH SLEEPING QUALITY SCALE

Buysse et al. developed the PSQS. The PSQS scale mainly evaluates the sleep quality, the amount of sleep, and sleep disturbances (18). The Turkish validity and reliability study of this study was conducted by Agargun et al. (19). The scale is composed of 19 questions and it is scored between 0 and 3. PSQS is composed of seven sub-dimensions such as subjective sleep quality, sleep latency, sleep duration, sleep adequacy, sleep disturbances, the use of sleeping pills, and days of dysfunction. High scores reflect the low sleep quality (18).

BIOCHEMICAL ANALYSIS

Venous blood samples of all individuals were collected into a dry tube after 10-12 hours of fasting and after 30 minutes of rest. All the blood samples were collected between 8 am and 9 am under the same conditions. Blood samples were centrifuged at 1000 g for 15 minutes right after they were taken. Serum samples were separated into different tubes and they were stored at -80 °C for the analyses. Complete blood count examined via flow cytometry technique (Mindray BC-6800 Auto Hematology Analyzer, Shenzhen, China); C-reactive protein (CRP), ALT and creatinine tests

were performed by spectrophotometric method (Abbot-Architect c8000, Japan); and erythrocyte sedimentation rate (ESR) was measured by Westergren method (Berkhum SDM-100, Turkey). Analyses were performed in the biochemistry laboratory of the Malatya State Hospital.

THIOL/DISULFIDE MEASUREMENT

Serum native thiol (NT), total thiol (TT) and disulfide levels ($\mu\text{mol/L}$) were measured by the method which was developed by Erel et al. (14) Disulfide/native thiol (DNT), disulfide/total thiol (DTT) and native thiol/total thiol (NTT) rates were calculated (%). In the presence of the sodium borohydride, disulfide bonds were reduced to functional thiol groups particularly. Then, the sodium borohydride was removed with the help of formaldehyde. Reduced and NT groups were measured after the 5,5'-dithiobis- (2-nitrobenzoic) acid (DTNB) reaction. (Disulfide amount was calculated by dividing the difference between TT and NT by two.

STATISTICAL ANALYSIS

The results were given as mean \pm SD and median (range). SPSS program (version 18, IBM, Chicago, IL, USA) was used for the statistical analysis. The homogeneity of the distribution of the groups was evaluated by Kolmogorov Smirnov test. Student's t-test was used for the data with normal distribution, the non-homogenous data such as ESR, CRP and pain duration were analyzed with Mann Whitney-U test. Categorical data were evaluated by chi-square test. Pearson correlation test was used for correlation analysis. A p value of < 0.05 was considered significant.

RESULTS

FIQ scores, number of sensitive points, and duration of pain were higher in FMS patients when compared to the controls ($p < 0.001$). In Table 1, sociodemographic characteristics, working status, medications, and clinical findings are given.

NT ($p = 0.005$) and NTT ($p < 0.001$) values of FMS patients were higher when compared to healthy controls. Similarly, disulfide ($p < 0.001$), DNT ($p < 0.001$) and DTT ($p < 0.001$) values were also higher in FMS patients compared to the controls. CRP and ESR values of FMS patients were found similar to the control group values. Thiol, disulfide and other biochemical findings of FMS patients are

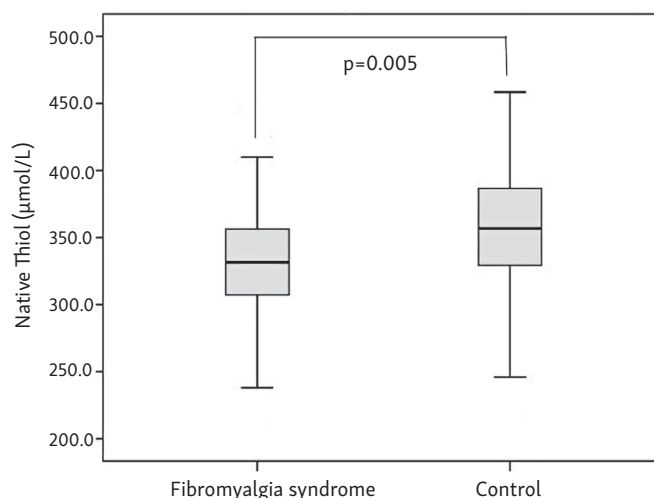


Fig. 1 Native thiol in Fibromyalgia syndrome.

Tab. 1 Demographic and clinic features of patients and control group.

	Fibromyalgia (n = 80) (mean \pm SD)	Control (n = 64) (mean \pm SD)	P value
Age (years)	41.4 \pm 7.6	39.9 \pm 10.9	0.341
BMI (kg/m ²)	26.7 \pm 3.7	26.5 \pm 5.6	0.811
Occupation (housewife/working) (n)	46/34	36/28	0.234
Pain duration (years) (median [range])	3.0 (0.5–20.0)	0.4 (0–9.0)	0.001
FIQ score	68.6 \pm 11.1	36.2 \pm 23.7	0.001
Number of sensitive points (n)	13.7 \pm 2.8	1.0 \pm 1.4	0.001
PSQS	7.5 \pm 3.7	3.8 \pm 3.2	0.001
SSRI (n)	33	0	0.001
MAOIs (n)	5	0	0.001
Pregabalin (n)	24	0	0.001
Gabapentin (n)	5	0	0.001
NSAIDs without other drugs (n)	13	0	0.001
NSAIDs with other drugs (n)	37	0	0.001
Opioids (n)	0	0	1.000
Cannabinoids (n)	0	0	1.000
Contraceptives (n)	0	0	1.000

Abbreviations: BMI – body mass index; FIQ – Fibromyalgia Impact Questionnaire; PSQS – Pittsburgh Sleeping Quality Scale; SSRI – selective serotonin reuptake inhibitor; MAOIs – monoamine oxidase inhibitors; NSAIDs – non-steroidal anti-inflammatory drugs.

Tab. 2 Results of thiol, disulfide and biochemical parameters in fibromyalgia and control groups.

	Fibromyalgia (n = 80) (mean ± SD)	Control (n = 64) (mean ± SD)	P value
Native Thiol (μmol/L)	330.6 ± 46.1	356.8 ± 55.5	0.005
Total Thiol (μmol/L)	369.5 ± 49.0	381.3 ± 51.3	0.183
Native thiol/total thiol ×100	89.4 ± 3.2	93.3 ± 4.0	0.001
Disulfide (μmol/L)	19.4 ± 6.3	12.2 ± 6.3	0.001
Disulfide/native thiol ×100	5.9 ± 2.0	3.6 ± 2.5	0.001
Disulfide/total thiol ×100	5.2 ± 1.6	3.3 ± 2.0	0.001
BUN (mg/dl)	12.9 ± 1.2	13.3 ± 1.1	0.062
Creatinine (mg/dl)	0.7 ± 0.4	0.7 ± 0.1	0.454
AST (IU/l)	17.9 ± 6.4	19.5 ± 8.0	0.201
ALT (IU/l)	17.4 ± 10.5	19.4 ± 9.7	0.237
HB (g/dl)	12.9 ± 1.2	13.3 ± 1.1	0.062
CRP (mg/dl) (median [range])	0.3 (0–14.0)	0.3 (0–4.3)	0.480
ESR (mm/h)	15.1 ± 8.3	15.5 ± 9.3	0.817

Abbreviations: BUN – blood urea nitrogen; AST – aspartate aminotransferase; ALT – alanine aminotransferase; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate.

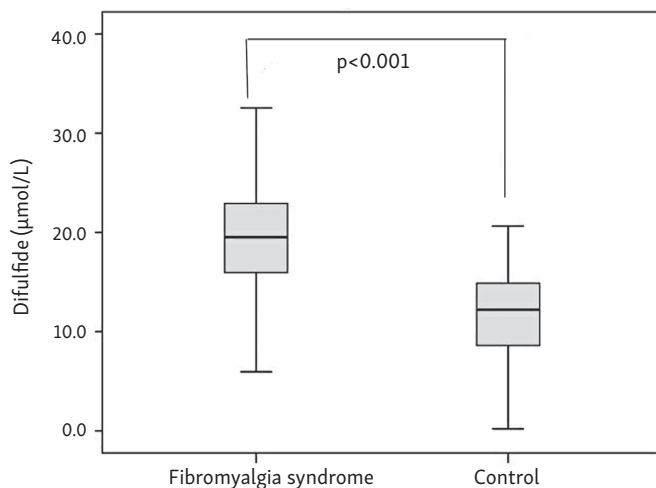
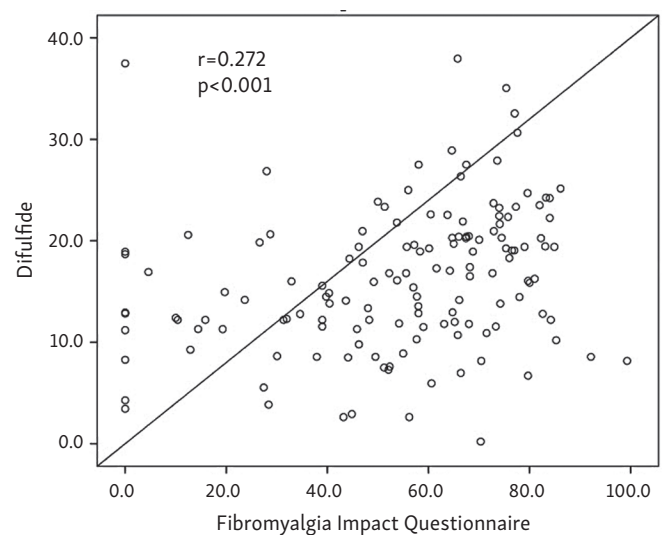
given in Table 2. The NT levels are shown in Figure 1 and disulfide levels are shown in Figure 2.

There was a positive correlation between FIQ and BMI ($r = 0.227$, $p = 0.006$) and age ($r = 0.240$, $p = 0.004$). In our study, there was not any correlation between CRP, ESR and FIQ, number of sensitive points, and duration of pain, thiol, and disulfide levels. There was a negative correlation between FIQ scores and NTT levels. We found a positive relationship between FIQ scores and disulfide, DNT and DTT values. There was a negative correlation between the number of sensitive points and NT and NTT levels while a positive correlation between D, DNT, and DTT was found. There was a positive relationship between the pain duration and disulfide values. FIQ scores, number of sensitive points and pain duration and thiol and disulfide levels had positive correlations. Results are given in Table 3. Correlation between disulfide and FIQ score is shown in Figure 3. Additionally, there was a negative correlation

between PSQS and NT ($r = -0.211$, $p = 0.012$), TT ($r = -0.170$, $p = 0.043$) and NTT ($r = -0.190$, $p = 0.023$). However, we found a positive correlation between PSQS scores and disulfide ($r = 0.166$, $p = 0.048$), DNT ($r = 0.172$, $p = 0.040$) and DTT ($r = 0.193$, $p = 0.021$).

DISCUSSION

The thiol levels of FMS patients were lower and the disulfide levels of patients were higher compared to control individuals. There was a significant relationship between thiol levels and FIQ scores, the number of sensitive points, and PSQS scores. FMS is commonly observed in women and it is characterized by a widespread pain. However, the etiology of FMS has not yet been clarified. FMS is associated with increased oxidative stress, widespread pain, fatigue and depression (20, 21). Chronic inflammation and

**Fig. 2** Disulfide levels in Fibromyalgia syndrome.**Fig. 3** Correlation between disulfide and FIQ score.

Tab. 3 Results of thiol, disulfide and other parameters for correlation analysis in fibromyalgia.

	FIQ score		Number of sensitive points		Pain duration	
	r value	p value	r value	p value	r value	p value
Native Thiol	-0.142	0.091	-0.267	0.001	-0.080	0.341
Total Thiol	-0.068	0.421	-0.133	0.113	-0.024	0.778
Native Thiol/Total Thiol	-0.240	0.004	-0.476	0.001	-0.164	0.051
Disulfide	0.272	0.001	0.492	0.001	0.204	0.015
Disulfide/Native Thiol	0.220	0.008	0.451	0.001	0.149	0.075
Disulfide/Total Thiol	0.240	0.004	0.475	0.001	0.164	0.050
Number of sensitive points	0.684	0.001			0.417	0.001
Pain Duration	0.396	0.001	0.417	0.001		

Abbreviations: FIQ, Fibromyalgia Impact Questionnaire.

oxidative stress can cause deterioration in the hypothalamic-pituitary-adrenal axis and thus FMS can occur (22). Oxidative stress can lead to mitochondrial damage and the deterioration of the energy balance and antioxidant defense system by affecting the protein kinases. Mitochondrial damage also takes place in FMS etiopathogenesis (23, 24). In the study of Neval et al., it was shown that total oxidant status and oxidative stress status were higher and total antioxidant status was lower in FMS patients when compared to the controls (25). The study by Fatima et al. showed that lipid peroxidation levels of FMS patients were higher and levels of antioxidant defense system enzymes such as glutathione peroxidase (GPx) and glutathione reductase in FMS patients were lower than the controls (21). However, there are studies stating that oxidant and antioxidant capacity of FMS patients were similar with the control group. Akbas et al. showed that GPx enzyme levels were similar in both FMS patients and healthy individuals (26, 27). Similarly, Toker et al. indicated that the ischemia-modified albumin levels were also similar in FMS patients and controls (26). Bozkurt et al. found that the oxidant status and oxidative stress index values of FMS patients were higher than controls but total antioxidant status and paraoxonase-1 levels were similar in patients and controls (28). Regarding these results, there are contradictory findings in the literature.

Glutathione is a tripeptide containing thiol and it protects the organism from the oxidative stress by activity of the enzyme GPx (29). In many studies, instead of studying the direct thiol group, glutathione and GPx levels were studied. Many drugs have a positive or negative effect on thiol levels (30). Our patients used selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), non-steroidal anti-inflammatory drugs (NSAIDs), pregabalin and gabapentin. SSRIs are found to increase GPx, glutathione and thiol levels in the brain tissue while they cause a severe decrease in GPx, glutathione and thiol levels in the liver tissue (31). It has been reported that pregabalin, gabapentin, and NSAIDs decrease GPx levels (30–33). The low level of GPx and glutathione indirectly indicates the low thiol levels. In this study, low thiol levels in FMS patients may be due to the use of these drugs.

It was shown by Sarifakioglu et al. that there was a strong relationship between the oxidative stress

parameters and FIQ, Beck depression inventory and visual analog scale scores (34). In our study, there was a strong positive relationship between thiol levels of FMS patients and their FIQ scores, number of sensitive points and the PSQS. On the other hand, we found a negative correlation between disulfide levels and FIQ scores, sensitive points, and the PSQS. Thiols have roles in the maintenance of the oxidative balance and they are molecules that contain sulfhydryl groups (10). Thiols create disulfide bonds in the presence of oxygen via oxidation (12). Thiols are mainly present in albumin and other protein derives such as cysteine, methionine, and reduced glutathione (35). Thiols have important roles in the clearance of hypochlorous acid from the body (36). It shows that oxidative stress increases due to the low thiol levels and high disulfide levels. The plasma thiol measurement was previously performed by using old manual methods (37). However, Erel et al. have recently found a cheap, easy, reliable, and fully automated method and the thiol/disulfide balance can be measured by using this technique (14). Using this method revealed that the balance of thiol/disulfide was deteriorated in favor of the disulfide in diseases such as Familial Mediterranean fever, cardiac disease and diabetes mellitus which are characterized with an enhanced oxidative stress (13, 38, 39). In our study, we showed that FMS patients have lower thiol levels and higher disulfide levels compared to controls. In FMS patients, there was a strong relationship between the number of sensitive points and the thiol/disulfide levels. Furthermore, the sleeping status and the thiol levels of the patients had a relationship. Our findings lead us to think that thiol/disulfide balance is deteriorated in FMS and oxidative stress has a role in the etiology of this disease.

In our study, CRP levels of our patients were not high and there was no correlation between the CRP levels and thiol levels of patients. CRP is a good indicator of inflammation. However, fibromyalgia is not an inflammation-related disease and CRP increase is not expected in the course (3). Xiao et al. showed that high sensitive (hs)-CRP levels were higher in FMS patients when compared to controls (40). However, patient and control groups were not matched regarding BMI-matched; FMS patients and controls had prominent weight difference. The group found that hs-CRP increased even though the

inflammation status was low. Rus et al. showed that both normal and the over-weighted FMS patients had higher CRP levels compared to the controls. However, a relationship was present between CRP levels and disease activity of the over-weighted FMS patients (41). Rus et al. had a smaller sample size and it is possible that the results might be affected due to the sample size. Xiao et al. and Rus et al. reported high CRP levels in obese FMS patients. It is well known that obesity causes inflammation. Therefore, studies of Xiao et al. and Rus et al. may found high CRP levels in FMS patients due to the obesity status of individuals rather than the disease. CRP is mostly secreted from the liver and CRP release is mainly induced by interleukin-6 (IL)-6. If the IL-6 release is low or if there is a lack of response against IL-6, CRP levels may not increase (42). It is known that IL-6 levels are not increased in FMS and both patients and the controls have similar levels of IL-6 cytokine (43, 44). In our study, we found similar levels of CRP in both the patient group and the control group most probably due to the lack of increase in IL-6 levels. Furthermore, increased cytokine level increases the oxidative stress but cytokine release is not required for the formation of the oxidative stress (45). This can be the reason why we could not reveal any relationship between CRP levels and thiol groups.

As a result, this study concluded that thiol levels were lower and disulfide levels were higher in FMS patients when compared to the controls. The change in the thiol/disulfide balance in favor of disulfide amounts reflects the oxidative stress. Increased oxidative stress can have a role in the deterioration of the thiol balance in FMS patients. It is possible to examine whether or not drugs with thiol groups have positive effects on FMS patients.

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