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Original Article

Effects of acupuncture on oxidative stress mechanisms, pain, and quality of life in fibromyalgia: A prospective study from Türkiye

Ezgi Aydın Özaslan[1], Fatma Gülçin Ural Nazlıkul[2], Gamze Avcıoğlu[3], Özcan Erel[4]

[1]Department of Physical Medicine and Rehabilitation, Ankara Bilkent City Hospital, Ankara, Türkiye
[2]Department of Physical Medicine and Rehabilitation, Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara, Türkiye
[3]Department of Medical Biochemistry, Karadeniz Ereğli State Hospital, Zonguldak, Türkiye
[4]Department of Medical Biochemistry, Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objectives: The aim of this study was to investigate the correlation between the use of real acupuncture and the quantities of intracellular oxidized, reduced, and total glutathione, as well as clinical indices including pain, depression, and quality of life in patients diagnosed with fibromyalgia syndrome (FMS).

Patients and methods: Between June 2019 and January 2020, a total of 52 female patients (mean age: 45.5±7.5 years; range, 30 to 56 years) who suffered from FMS and 26 healthy females (mean age: 44.2±6.8 years; range, 29 to 52 years) were included in a prospective manner. The patients were divided into two groups: those who received real acupuncture (n=26) and those who received sham acupuncture (n=25). The clinical features of the subjects were assessed at three time points: before therapy (T0), after the last session of treatment (T1), and one month following intervention (T2). The levels of intracellular oxidized, reduced, and total glutathione were assessed in whole sample at two time points, T0 and T2.

Results: At time T0, the quantity of intracellular oxidized, reduced, and total glutathione were higher in FMS patients than the control group, indicating higher levels of oxidative stress (p=0.001). In the group that received real acupuncture, there was a notable increase in the levels of intracellular oxidized, reduced, and total glutathione in T2 compared to T0. The difference in antioxidant activity was statistically significant (p=0.001). While comparing the percentage alterations in clinical variables and oxidative stress indicators between the real and sham groups at T0 and T2, the differences in the real acupuncture group were much higher (p=0.001).

Conclusion: Our study results indicate that real acupuncture may have an effect on the oxidative homeostasis in individuals with FMS.

Keywords: Acupuncture, fibromyalgia, glutathione, oxidative stress.

Fibromyalgia syndrome (FMS) is a chronic disease of unknown etiology, typically characterized by symptoms such as widespread pain, fatigue, headache, irritable bowel syndrome, memory impairments and mood disorders.[1] It has been reported that the global mean prevalence of the FMS is 2.7% and the prevalence reaches the highest level between the ages of 30 and 50.[2] Considering the pathophysiology of FMS, it is thought that the insufficiency of the central nervous system in pain inhibition may cause abnormal modulation of sensory inputs and this may cause pain.[3] Although the etiopathogenesis of FMS has not been clearly elucidated, it has been shown that many factors are correlated and it has been accepted as a multifactorial disease.[4] Basically, genetic factors, central and peripheral theories, psychological factors, immunological mechanisms and oxidative stress are involved in the etiopathogenesis of FMS.[5]

Oxidative stress arises from the disparity between the production of free radicals and the capacity of the organism to defend against them with antioxidants. This leads to the impairment of many molecular components such as fats, proteins, and nucleic acids.[6] It can result in cellular

Corresponding author: Ezgi Aydın Özaslan, MD. Ankara Yıldırım Beyazıt Üniversitesi, Ankara Bilkent Şehir Hastanesi, Fiziksel Tıp ve Rehabilitasyon Kliniği, 06800 Çankaya, Ankara, Türkiye.
E-mail: drezgiaydin@gmail.com

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breakdown which leads to the pathophysiology of several disorders, including FMS. An increased oxidative and nitrosative stress level is thought to accompany the chronic proinflammatory condition, which is involved in the etiology of FMS.[7] Studies on the pathophysiology of FMS indicate that the deterioration in oxidant/antioxidant balance and the increase in free radical levels may play a role in the disease process.[8-11] In addition, oxidative stress has been shown to play a role in pain, muscle fatigue symptoms and accompanying depression in FMS.[12,13]

Glutathione, a non-protein antioxidant that contains the thiol group, is the most abundant in the cell and has an antioxidant role. The oxidized and reduced forms of glutathione indicate the oxidant/antioxidant status of the cell. Oxidized glutathione (GSSG) is formed as a result of the reduction of lipid hydroperoxides, and the increase in GSSG concentration is accepted as an indicator of oxidative stress.[14] The GSSG is converted to reduced glutathione (GSH) by the GSH reductase enzyme and GSH is a highly abundant and crucial antioxidant found in red blood cells.[15] It has been reported that low GSH levels are positively associated with the presence of increased oxidative stress in chronic fatigue syndromes such as FMS and depression.[16] Low GSH level causes impairment in intracellular signal transmission. This may result in a significant increase in intracellular free radical levels and oxidative stress-mediated damage to mitochondrial deoxyribonucleic acid (DNA). As a result, mitochondrial function may be impaired. Since skeletal muscle and brain cells have high metabolic rates and are very sensitive to ATP deprivation, exercise capacity may decrease and fatigue may increase.[17] Therefore, glutathione mechanisms should be investigated in the pathogenesis of FMS.

Acupuncture is among the first step of non-pharmacological treatment options in FMS.[18] It demonstrates that the stimulation of specific acupuncture sites can influence the release of pain-relieving compounds in the brain and local region, such as serotonin and norepinephrine, which leads to an alleviation of pain experiences.[19] Acupuncture may cause an increase in the levels of opioid peptides such as serotonin and endorphins in the cerebrospinal fluid (CSF).[20] In addition, current studies have shown that acupuncture has the effects of antagonizing oxidative stress in diseases such as dementia, Parkinson's and Alzheimer's, either directly or indirectly, by preventing the reaction of free radicals, increasing superoxide dismutase (SOD) activity and reducing lipid peroxide level.[21]

Although acupuncture is widely used in the treatment of FMS, it is not known what effect it has on oxidative stress, which has been shown to play a role in FMS etiopathogenesis in recent studies. Intra-erythrocyte oxidized, reduced and total GSH levels reflect the state of intracellular oxidative stress. In the light of this information, in the first step of this study, intracellular oxidized (GSSG), reduced (GSH) and total glutathione (total GSH) levels were investigated in females diagnosed with FMS. In the second step, possible effects of acupuncture treatments on these biomarkers and, as well as clinical indices including pain, depression and quality of life (QoL) were evaluated.

In the present study, we hypothesized that oxidative stress is one of the etiological factors for FMS and that acupuncture applications can reduce oxidative stress together with the clinical parameters of FMS. We, therefore, aimed to investigate whether there was a change in these parameters after acupuncture and if there was a change, in what direction it was affected.

PATIENTS AND METHODS

Study design and study population

This single-blind case-control study was conducted at Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara City Hospital, Department of Physical Medicine and Rehabilitation between June 2019 and January 2020. Female patients with FMS and female controls were recruited. Patients who were diagnosed with FMS according to the 2016 American College of Rheumatology (ACR) criteria for fibromyalgia and who did not accept pharmacological treatment were included in this study. Exclusion criteria included patients who underwent acupuncture therapy in the past for any reason, individuals with a medical history including malignancy, nicotine and alcohol use, insufficient vitamin D levels, or a history of neurological, orthopedic, rheumatic, or psychiatric diseases. A total of 52 female patients (mean age: 45.5±7.5 years; range, 30 to 56 years) who met the inclusion criteria were enrolled. The patients were divided into two equal groups by computer-based randomization as real acupuncture and sham acupuncture groups. A control group was formed with 26 age-and sex-matched volunteers (mean age: 44.2±6.8 years;

range, 29 to 52 years) who did not have exclusion criteria. The patients were not informed about group allocation and it was ensured that the study was a single-blind study. Written informed consent was obtained from each patient. The study protocol was approved by the Yıldırım Beyazıt University Faculty of Medicine Clinical Research Ethics Committee (date: 06.02.2019., no: 46). The study was conducted in accordance with th.e principles of the Declaration of Helsinki.

Blood samples and determination of intracellular oxidized, reduced and total GSH levels

For the measurement of intracellular GSSG, GSH, and total GSH levels among oxidative stress markers, 3 to 5 mL whole blood sample was taken into commercially available ethylenediamine tetraacetic acid (EDTA)-treated tubes. Blood samples were taken once from the control group and twice from the patient group, before the treatment (T0) and one month after the treatment (T2). The EDTA-treated tubes were centrifuged at 900 g at +4°C for 10 min with a cooled centrifuge at the Biochemistry Laboratory after a 30-min incubation period. Intracellular GSSG, GSH, and total GSH levels were measured by the spectrophotometric method developed by Alisik et al.[22] After centrifugation, the supernatant was divided in half, GSH was measured in the first part and total GSH (GSH + GSSG) was measured in the second part. The GSH content was subtracted from the total GSH (GSH + GSSG) content and divided by two equals to the GSSG amount. The results were shown as µmol/L. The GSSG/GSH percentage ratios were calculated.[22]

Intervention

The intervention was planned as three sessions per week (10 sessions in total), 20 min per session for each patient. The same acupuncture points were chosen for all patients without modification for the specific symptoms of the patient. The acupuncture points employed were CV 6, GV 20, EX-HN 3 and bilaterally Pc 6, H 7, LI 4, CV 17, LIV 3, ST 36, SP 6, SI 3, GB 34. These points were chosen[23-26] to relieve and treat the symptoms seen in FMS were applied by same physician, who has an acupuncture application certificate. The Pc 6 and H 7 are used in the treatment of sleep disorders, depression, dizziness, and upper extremity pain. The LI 4 is an important pain relief point. It has effects on digestive system disorders, headaches, and the immune system. The CV 6 has a supportive effect on general fatigue and weakness. The CV 17 is the junction of respiratory energy. It is also used in anxiety. The LIV 3 is effective in sleep disorders, chronic constipation, and gastrointestinal problems. The ST 36 is for gastrointestinal problems, sleep disorders, lower extremity pain. The SP 6 is effective in the immune system, sleep disturbance, pain, and numbness in the lower extremity. The SI 3 is for neck-low back pain, wrist pain and tinnitus. The GB 34 is used for knee, neck, shoulder pain and headache. The GV 20 is effective on sleep disturbance, headache, and memory impairment. The EX-HN 3 is used for headaches, sleep disorders and psychological problems. It is thought to improve well-being.[27] Disposable 0.25×25-mm acupuncture needles were used in the real acupuncture group. The needle was inserted without extra rotational or manual stimulation, and depth of needle penetration was determined by the patient's sensitivity until a feeling of chi was obtained. The needles were inserted into the acupuncture points, while the patients were in the supine position. The inclination of the needle was 90° at all points. Park sham devices were used in the sham acupuncture group. This device is a non-penetrating needle device with a blunt retractable needle and guide tube. After adhering the guide tube to the skin using a self-adhesive material, it is carefully pressed to the designated location. Subsequently, a cautious insertion of the blunt sham needle into the guide tube occurs. By gently tapping the top of the needle, it is allowed to move through the tube, so that the needle feels like a prick without penetrating the skin. During this process, there is no rotational or manual stimulation.

Procedures

This study was conducted in three consecutive phases:

T0: Before intervention

T1: At the end of intervention

T2: Considering that the maximum efficiency of acupuncture was seen after four weeks on average,[23] it was four weeks after the end of intervention.

In the first phase of this study (T0), sociodemographic data were filled in with the information received from the all participants. A total of 5 mL of venous blood samples were taken from all participants to determine the level of intracellular oxidative stress. The patient groups were randomly divided into real acupuncture and sham acupuncture groups determined as single-blind. Data on clinical parameters were

obtained from the both patient groups as indicated in the sentences below. The Visual Analog Scale (VAS) was used to evaluate the pain intensity of the patients during activity, rest and night. Fatigue severity of the patients was evaluated with the Fatigue Severity Scale (FSS). In this study, it was used to differentiate fatigue from clinical depression. The Turkish validity and reliability study was conducted in 2007 by Armutlu et al.[24] The Fibromyalgia Impact Questionnaire (FIQ) was used to evaluate the functional status of our patients with FMS, disease progression and outcomes. High scores on the FIQ indicate a decrease in functionality. The Turkish validity and reliability study was conducted by Sarmer et al.[28] The Beck Depression Inventory (BDI) was used to determine depression levels of our sample. It is a self-report scale composed of 21 multiple-choice questions. Over 17 points is considered to be depression. The Turkish validity and reliability study was conducted by Hisli[29] The Short Form-36 (SF-36) was used to evaluate the QoL of our patients. This scale has eight subscales: physical functioning, role limitations due to physical health, role limitations due to emotional problems, energy/fatigue, emotional well-being, social functioning, pain and general health. Scores between 0-100 can be obtained from each scale, high scores indicate an improved QoL. The Turkish validity and reliability study was conducted by Koçyiğit et al.[30] According to the intervention method determined for the groups, the intervention was applied to the patients by the same physician who was authorized to perform acupuncture with the approval of the Republic of Türkiye, Ministry of Health.

In the second phase of the study (T1), the VAS, FIQ, BDI, SF-36, and FSS scales were administered a second time to evaluate clinical data of both real and sham acupuncture groups at the end of the intervention.

In the last phase of the study, four weeks after the end of the treatment (T2), to evaluate the effects of real and sham acupuncture applications on the oxidative stress level in patients with FMS, venous blood samples were taken again. The VAS, FIQ, BDI, SF-36, and FSS scales were administered to assess clinical data of both real and sham acupuncture groups four weeks after the end of the treatment.

Statistical analysis

Power analysis and sample size calculation were performed using the G*Power version 3.1.9.7 software (Heinrich-Heine-Universität, Düsseldorf, Düsseldorf, Germany). It was determined that 23 participants per group would be sufficient to detect a significant difference with an effect size of 0.25 at 80% power and a 0.05 alpha error level.

Statistical analysis was performed using the SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). The normality of numerical variables was evaluated through the examination of skewness and kurtosis values and indices.[31] The findings, including descriptive statistics and normality test results, are presented in supplementary material (S1). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency. The chi-square (χ^2) test was used to compare qualitative data. The Mann-Whitney U test was used to compare scale scores and the Friedman test was used to compare repeated measures. The independent samples t-test or one-way analysis of variance (ANOVA) was used for the evaluation of quantitative data with normal distribution. The paired samples t-test was used for the comparison of repeated measurements.

TABLE 1
Comparison of demographic and clinical characteristics between groups

	Real acupuncture group (n=26)			Sham acupuncture group (n=25)			Control group (n=26)			
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (year)			45.2 ± 8.0			45.8 ± 7.1			44.2 ± 6.8	0.254[a]
Disease duration (year)			4.2 ± 2.0			3.9 ± 1.1			--	0.601[b]
Body mass index (kg/m^2)			27.3 ± 2.0			26.8 ± 2.5			27.0 ± 0.9	0.624[a]
Occupation										0.839[c]
Nonemployee	18	69.2		16	64.0		16	61.5		
Employee	8	30.8		9	36.0		10	38.5		

SD: Standard deviation; a: One-way ANOVA; b: Independent samples t test; c: Pearson chi-square test.

TABLE 2
Comparison of clinical parameters within and between groups

			Real acupuncture group (n=26)		Sham acupuncture group (n=25)		
			Median	Min-Max	Median	Min-Max	*p*
VAS	Night	T0	4.0	3.0-5.0	4.0	3.0-5.0	0.057[a]
		T1	2.0	1.0-2.0	2.0	1.0-2.0	
		T2	2.0	1.0-2.0	2.0	1.0-2.0	
		*p**	0.001[b]		0.001[b]		
		T0-T1	0.001[c]		0.001[c]		
		T0-T2	0.001[c]		0.258[c]		
		T1-T2	0.405[c]		0.001[c]		
	Rest	T0	5.0	4.0-6.0	4.0	4.0-5.0	0.055[a]
		T1	2.0	2.0-3.0	2.0	2.0-3.0	
		T2	2.0	2.0-3.0	4.0	3.0-4.0	
		*p**	0.001[b]		0.001[b]		
		T0-T1	0.001[c]		0.001[c]		
		T0-T2	0.001[c]		0.085[c]		
		T1-T2	0.677[c]		0.001[c]		
	Activity	T0	8.0	7.8-8.0	8.0	7.0-8.0	0.610[a]
		T1	4.0	3.0-5.0	5.0	4.5-5.0	
		T2	4.0	3.0-4.0	7.0	6.0-7.0	
		*p**	0.001[b]		0.001[b]		
		T0-T1	0.001[c]		0.001[c]		
		T0-T2	0.001[c]		0.002[c]		
		T1-T2	0.579[c]		0.001[c]		
FIQ		T0	67.51	58.15-79.57	65.97	60.0-72.75	0.888[a]
		T1	39.72	30.94-46.48	49.92	42.49-56.35	
		T2	39.77	30.94-47.77	56.87	50.06-60.72	
		*p**	0.001[b]		0.001[b]		
		T0-T1	0.001[c]		0.001[c]		
		T0-T2	0.001[c]		0.001[c]		
		T1-T2	0.096[c]		0.001[c]		
FSS		T0	5.33	5.0-6.0	5.44	4.88-6.0	0.962[a]
		T1	3.66	3.0-4.33	4.22	4.0-4.88	
		T2	3.77	3.11-4.44	4.44	4.0-5.11	
		*p**	0.001[b]		0.001[b]		
		T0-T1	0.001[c]		0.001[c]		
		T0-T2	0.001[c]		0.001[c]		
		T1-T2	0.332[c]		0.034[c]		
BDI		T0	18.0	13.0-20.0	17.0	12.0-21.0	0.760[a]
		T1	10.0	6.0-15.0	13.0	9.0-17.0	
		T2	10.0	6.0-14.0	14.0	11.0-18.0	
		*p**	0.001[b]		0.001[b]		
		T0-T1	0.001[c]		0.001[c]		
		T0-T2	0.001[c]		0.001[c]		
		T1-T2	0.579[c]		0.011[c]		

TABLE 2
Continued

			Real acupuncture group (n=26)		Sham acupuncture group (n=25)		
			Median	Min-Max	Median	Min-Max	*p*
SF-36	Physical functioning	T0	45.0	20.0-75.0	50.0	20.0-65.0	0.433[a]
		T1	62.5	45.0-80.0	65.0	55.0-75.0	
		T2	45.0	20.0-75.0	50.0	20.0-65.0	
		*p**		0.001[b]		0.001[b]	
		T0-T1		0.001[c]		0.001[c]	
		T0-T2		0.001[c]		0.024[c]	
		T1-T2		0.032[c]		0.002[c]	
	Role limitations due to physical health	T0	25.0	0.0-75.0	50.0	0.0-50.0	0.554[a]
		T1	75.0	50.0-100.0	75.0	25.0-100.0	
		T2	50.0	5.0-100.0	50.0	25.0-75.0	
		*p**		0.001[b]		0.001[b]	
		T0-T1		0.001[c]		0.001[c]	
		T0-T2		0.001[c]		0.198[c]	
		T1-T2		0.071[c]		0.003[c]	
	Role limitations due to emotional problems	T0	66.7	0.0-66.7	33.3	33.3-66.7	0.898[a]
		T1	66.7	33.3-100.0	66.7	33.3-100.0	
		T2	66.7	33.3-100.0	66.7	33.3-100.0	
		*p**		0.001[b]		0.001[b]	
		T0-T1		0.001[c]		0.011[c]	
		T0-T2		0.002[c]		0.525[c]	
		T1-T2		0.298[c]		0.056[c]	
	Energy/fatigue	T0	30.0	25.0-45.0	35.0	30.0-45.0	0.123[a]
		T1	60.0	45.0-70.0	55.0	40.0-65.0	
		T2	55.0	40.0-65.0	45.0	35.0-65.0	
		*p**		0.001[b]		0.001[b]	
		T0-T1		0.001[c]		0.001[c]	
		T0-T2		0.001[c]		0.001[c]	
		T1-T2		0.002[c]		0.017[c]	
	Emotional well-being	T0	48.0	32.0-64.0	52.0	40.0-60.0	0.560[a]
		T1	64.0	52.0-72.0	64.0	52.0-72.0	
		T2	62.0	48.0-72.0	60.0	48.0-72.0	
		*p**		0.001[b]		0.001[b]	
		T0-T1		0.001[c]		0.001[c]	
		T0-T2		0.002[c]		0.001[c]	
		T1-T2		0.027[c]		0.013[c]	
	Social functioning	T0	50.0	25.0-62.5	50.0	25.0-62.5	0.557[a]
		T1	62.5	37.5-75.0	50.0	50.0-75.0	
		T2	62.5	50.0-75.0	50.0	50.0-75.0	
		*p**		0.001[b]		0.001[b]	
		T0-T1		0.001[c]		0.016[c]	
		T0-T2		0.001[c]		0.104[c]	
		T1-T2		1.000[c]		0.437[c]	

TABLE 2
Continued

			Real acupuncture group (n=26)		Sham acupuncture group (n=25)		
			Median	Min-Max	Median	Min-Max	*p*
SF-36	Pain	T0	22.5	10.0-45.0	22.5	10.0-55.0	0.789[d]
		T1	45.0	45.0-67.5	45.0	2.5-57.5	
		T2	45.0	45.0-67.5	32.5	22.5-45.0	
		*p**		0.001[b]		0.001[b]	
		T0-T1		0.001[c]		0.001[c]	
		T0-T2		0.001[c]		0.040[c]	
		T1-T2		0.488[c]		0.001[c]	
	General health	T0	50.0	35.0-65.0	45.0	35.0-60.0	0.782[a]
		T1	55.0	45.0-70.0	55.0	40.0-65.0	
		T2	55.0	40.0-65.0	50.0	40.0-60.0	
		*p**		0.005[b]		0.001[b]	
		T0-T1		0.004[c]		0.002[c]	
		T0-T2		0.127[c]		0.621[c]	
		T1-T2		0.188[c]		0.009[c]	

VAS: Visual Analog Scale; FIQ: Fibromyalgia impact questionnaire; FSS: Fatigue severity scale; BDI: Beck depression inventory; SF-36: Short Form-36; The significance value was set to * p<0.017; a: Mann-Whitney U test; b: Friedman test; c: Adjusted p-values using the Bonferroni correction; T0: Before treatment; T1: At the end of treatment; T2: At the first month after treatment.

To control for the risk of type 1 error due to multiple comparisons, Bonferroni tests were used to find the source of the difference in cases where differences were found in multiple comparisons. A *p* value of <0.05 was considered statistically significant and the threshold for statistical significance was adjusted to p<0.017 for analyses involving Bonferroni corrections.

RESULTS

The demographic characteristics of the participants are shown in Table 1. The age (p=0.254), body mass index (p=0.624), and occupation (p=0.839) variables did not significantly differ between the groups.

Comparison of factors associated with FMS of the both acupuncture groups at T0

The VAS, FIQ, FSS, BDI and SF-36 scores are shown in Table 2. There was no statistically significant difference between real and sham acupuncture groups in terms of the scores of VAS night (p=0.057), VAS rest (p=0.055), VAS activity (p=0.610), FIQ (p=0.888), FSS (p=0.962), BDI (p=0.760), and SF-36 physical functioning (p=0.433), role limitations due to physical health (p=0.554), role limitations due to emotional problems (p=0.898), energy/fatigue (p=0.123), emotional well-being (p=0.560), social functioning (p=0.557), pain (p=0.789), and general health (p=0.782) at T0.

Comparison of the change in FMS-related clinical parameters of both acupuncture groups at T0-T1-T2

The intra-group difference in the VAS, FIQ, FSS, BDI and SF-36 scores obtained at T0-T1 and T2 time points of real and sham acupuncture groups are summarized in Table 2. To identify the specific time point at which these differences emerged, comparisons between the two groups were conducted with the application of the Bonferroni correction to control for multiple testing. Results from the Friedman test indicated statistically significant differences in the VAS, FIQ, FSS, BDI, and SF-36 scores between the T0-T1 and T2 time points for both real and sham acupuncture groups. Notably, all p-values were found to be 0.001, except for the general health subscale of the SF-36, which was 0.005. In the real acupuncture group, significant reductions were observed in the VAS, FIQ, FSS, and BDI scores at T1 compared to T0, with all initial reductions marked by p-values of 0.001. The comparisons between T1 and T2 did not reveal significant differences for these measures (VAS night p=0.405, VAS rest p=0.677, VAS activity p=0.579,

FIQ p=0.096, FSS p=0.332, and BDI p=0.579). In the sham acupuncture group, the VAS night score at T1 was significantly lower compared to both T0 and T2 (p=0.001, p=0.001), whereas the VAS night score between T0 and T2 did not significantly differ (p=0.285). Additionally, significant differences were observed in the rest and activity subscales of VAS (with the exception of the rest score from T0 to T2, p=0.085), as well as in FIQ (with the exception of the rest score from T1 to T2, p=0.034), FSS, and BDI scores, across any two time points. All p-values were 0.001, with the exception of the VAS activity score from T0 to T2 and the BDI score from T1 to T2, which were p=0.002 and p=0.011, respectively. Notably, these scores were the highest at T0 and the lowest at T1.

In the real acupuncture group, significant improvements were noted across all SF-36 subscales at T1 compared to T0, with all improvements demonstrating p-values of 0.001, except for the general health subscale, which showed a significant improvement at p=0.005. Furthermore, significant improvements were noted across all SF-36

TABLE 3
Comparison of the percentage changes between groups of clinical parameters

			Real acupuncture group (n=26)		Sham acupuncture group (n=25)		
			Median	Min-Max	Median	Min-Max	*p*"
VAS	Night	T0-T1	60.0	33.3-100.0	33.3	0.0-50.0	**0.001**
		T0-T2	60.0	0.0-75.0	0.0	0.0-50.0	**0.001**
	Rest	T0-T1	50.0	25.0-71.4	40.0	20.0-60.0	**0.001**
		T0-T2	50.0	25.0-75.0	20.0	0.0-33.3	**0.001**
	Activity	T0-T1	50.0	28.6-62.5	37.5	28.6-50.0	**0.001**
		T0-T2	50.0	37.5-62.5	12.5	0.0-25.0	**0.001**
FIQ		T0-T1	39.8	24.4-57.0	23.3	16.9-41.6	**0.001**
		T0-T2	38.5	22.8-56.4	14.6	10.2-30.6	**0.001**
FSS		T0-T1	31.3	17.0-43.7	20.0	13.4-30.7	**0.001**
		T0-T2	29.5	14.9-41.7	19.2	6.1-27.4	**0.001**
BDI		T0-T1	38.7	22.2-62.5	21.1	11.8-47.4	**0.001**
		T0-T2	39.5	26.3-60.0	15.4	5.6-36.8	**0.001**
SF-36	Physical functioning	T0-T1	34.9	6.7-175.0	27.3	0.0-200.0	0.350
		T0-T2	22.2	0.0-200.0	18.2	0.0-200.0	0.444
	Role limitations due to physical health	T0-T1	100.0	0.0-200.0	100.0	0.0-300.0	0.724
		T0-T2	50.0	0.0-200.0	0.0	0.0-200.0	**0.030**
	Role limitations due to emotional problems	T0-T1	49.3	0.0-103.0	49.3	0.0-203.0	0.799
		T0-T2	49.3	0.0-103.0	0.0	0.0-103.0	**0.033**
	Energy/fatigue	T0-T1	91.7	44.4-160.0	62.5	0.0-116.7	**0.015**
		T0-T2	58.6	25.0-120.0	28.6	0.0-116.7	**0.001**
	Emotional well-being	T0-T1	28.6	6.3-75.0	23.1	0.0-80.0	0.322
		T0-T2	24.4	0.0-75.0	15.4	0.0-40.0	**0.044**
	Social functioning	T0-T1	50.0	0.0-100.0	0.0	0.0-100.0	**0.008**
		T0-T2	50.0	0.0-100.0	0.0	0.0-100.0	**0.001**
	Pain	T0-T1	95.7	28.6-350.0	95.7	0.0-350.0	0.500
		T0-T2	95.7	22.2-350.0	43.5	0.0-350.0	**0.001**
	General health	T0-T1	10.0	0.0-50.0	10.0	0.0-33.3	0.732
		T0-T2	9.1	0.0-28.6	11.1	0.0-33.3	0.454

VAS: Visual Analog Scale; FIQ: Fibromyalgia Impact Questionnaire; FSS: Fatigue Severity Scale; BDI: Beck Depression Inventory; SF-36: Short Form-36; T0: Before treatment; T1: At the end of treatment; T2: At the first month after treatment; * Mann-Whitney U test was used.

subscales, except for the general health subscale at T2 compared to T0, with all improvements demonstrating p-values of 0.001 except for the role limitations due to emotional problems and emotional well-being subscales which showed a significant improvement at p=0.002. Application of the Bonferroni adjustment for the general health subscale to comparisons between T0 and T2 showed no significant differences (p=0.127). According to Bonferroni adjustment comparisons between T1 and T2, there were no significant differences for all of the SF-36 subscales except the energy/fatique subscale. The significant reductions were noted at T2 compared to T1 in the scores for energy/fatigue (p=0.002).

In the sham acupuncture group, significant improvements were noted across all SF-36 subscales at T1 compared to T0, with all improvements demonstrating p-values of 0.001, except for the role limitations due to emotional problems, social functioning and general health subscales (p=0.011, p=0.016, and p=0.002, respectively). While comparisons between T0 and T2 revealed no significant differences for physical functioning (p=0.024), role limitations due to physical health (p=0.198), role limitations due to emotional problems (p=0.525), social functioning (p=0.104), pain (p=0.040), general health (p=0.621), energy/fatigue (p=0.001), and emotional well-being (p=0.001) subscales showed a significant improvement. However, significant reductions were noted at T2 compared to T1 in the scores for physical functioning (p=0.002), role limitations due to physical health (p=0.003), energy (p=0.017), emotional well-being (p=0.013), pain (p=0.001), and general health (p=0.009).

Comparison of the percentage change in FMS-related clinical parameters of between acupuncture groups at T0-T1 and T0-T2

We investigated whether there were any differences in the percentage change of clinical parameters between the real and sham acupuncture groups at time points T0-T1 and T0-T2. It was determined that the percentage change in the VAS night, activity and rest scores in both time intervals were significantly higher in the real acupuncture group compared to the sham acupuncture group. The percentage changes of FIQ, FSS and BDI scores were also found to be significantly higher in the real acupuncture group compared to the sham acupuncture group in both time intervals. When the percentage change of SF-36 subscales were compared, the percentage change in role limitations due to physical health, role limitations due to emotional problems, emotional well-being and pain in T0-T2 time interval, and energy and social function in both time intervals were significantly higher in the real acupuncture group than in the sham acupuncture group (Table 3).

Comparison of oxidative stress parameters between the patients with FMS and control groups at T0

The GSH (p=0.001) and total GSH (p=0.001) values were higher in the control group compared to the

TABLE 4
Comparison of intracellular oxidized, reduced and total glutathione levels within and between groups

		Real acupuncture group (n=26)	Sham acupuncture group (n=25)	Control group (n=26)	
		Mean±SD	Mean±SD	Mean±SD	*p*
GSH (µmol/L)	T0	645.1±49.0	637.8±46.5	825.1±92.6	**0.001**[a*]
	T2	765.3±47.1	631.2±20.7	-	
	p	0.001[b]	0.472[b]	-	
GSH + GSSG (µmol/L)	T0	869.4±49.5	866.0±47.1	990.5±101.5	**0.001**[a*]
	T2	928.8±44.1	867.8±23.4	-	
	p	0.001[b]	0.831[b]	-	
GSSG (µmol/L)	T0	112.2±7.1	114.1±4.7	82.7±14.2	**0.001**[a*]
	T2	81.8±9.4	118.3±5.3	-	
	p	0.001[b]	0.001[b]	-	

SD: Standard deviation; GSH: Reduced glutathione; GSSG: Oxidized glutathione; T0: Before treatment; T2: At the first month after treatment; * The difference is between the control group and real-sham acupuncture groups; a: One-Way Anova test; b: Paired Samples t test.

TABLE 5
Comparison of percentage changes of intracellular oxidized, reduced and total glutathione levels between groups

		Real acupuncture group (n=26)	Sham acupuncture group (n=25)	
		Mean±SD	Mean±SD	*p*
GSH (µmol/L)	T0-T2	19.1±10.1	-1.0±7.6	**0.001**
GSH + GSSG (µmol/L)	T0-T2	7.2±7.6	-0.4±5.1	**0.001**
GSSG (µmol/L)	T0-T2	-26.7±10.3	3.8±5.3	**0.001**

SD: Standard deviation; GSH: Reduced glutathione; GSSG: Oxidized glutathione; T0: Before treatment; T2: At the first month after treatment; Independent Samples t test was used.

patients with FMS and GSSG (p=0.001) values were lower in the control group than in the patients with FMS (Table 4).

Comparison of oxidative stress parameters of the both acupuncture groups at T0 and T2 times

In the real acupuncture group, GSH (p=0.001) and total GSH (p=0.001) values were found to be higher at T2 compared to T0, while GSSG (p=0.001) values were lower at T2 than T0. GSSG (p=0.001) values were higher at T2 than T0 in the sham acupuncture group. There was no significant difference between the times (T0-T2) in the sham acupuncture group in terms of GSH (p=0.472) and total GSH (p=0.831) values (Table 4).

Comparison of oxidative stress parameters between real acupuncture and sham acupuncture groups at T2

When the percentage change of glutathione levels, it was found that the percentage change in GSH (p=0.001), total GSH (p=0.001), and GSSG (p=0.001) values of the patients in the real acupuncture group was higher than those in the sham acupuncture group in T0-T2 time interval (Table 5).

DISCUSSION

In this study, we investigated whether there was a change in these parameters after acupuncture and if there was a change, in what direction it was affected. Our study showed that female patients with FMS had higher intracellular GSSG levels and lower GSH and total GSH levels compared to healthy controls. While assessing the impact of real and sham acupuncture interventions on oxidative stress, all the indicators examined in the real acupuncture group showed a decline in oxidative stress throughout the first month following interventions, as opposed to prior intervention. However, interestingly, in the first month after sham acupuncture, there was a change in GSSG levels in the direction of increased oxidative stress compared to before treatment. In addition, GSH and total GSH levels did not change in the first month after treatment in the sham acupuncture group compared to before treatment. To the best of our knowledge, this is the first study to investigate the effects of acupuncture on oxidative stress biomarkers in patients with FMS.

In the present study, differences were found in intracellular GSH, GSH+GSSG and GSSG levels between real acupuncture, sham acupuncture and control groups. These results may suggest that the antioxidant/oxidant balance, as assessed by intracellular GSH/GSSG, is deteriorated to the oxidant side in patients with FMS compared to healthy controls. Numerous researches in the literature have examined the roles of oxidative stress in the pathophysiology of FMS. In a study conducted by Şendur et al.,[10] catalase and glutathione peroxidase (GPx), which are important antioxidants, were significantly lower in patients with FMS, emphasizing that these antioxidants are important in the pathophysiology of FMS and other neurological disorders. The data obtained from our study support the results of studies suggesting that oxidative stress increases in FMS and dysfunction in the glutathione system.[16,17,32,33] In our study, intracellular oxidized, reduced and total glutathione biomarkers, which are currently used in determining the oxidative stress, were used in FMS for the first time. Findings suggest that measuring intracellular levels of oxidized, reduced, and total glutathione may be useful in identifying and managing FMS, in which oxidative stress plays a role in the etiology. Therefore, our results should be supported by randomized-controlled studies to be conducted with larger samples.

In the real acupuncture group, the percentage changes of GSH and total GSH levels, which are antioxidant markers, increased significantly, and the percentage changes of GSSG levels, which is oxidant marker, decreased in the first month after treatment. Within the sham acupuncture group, GSSG levels exhibited an increase during the initial month following treatment, whereas we had any notable alteration in GSH and total GSH levels. According to these results, it can be suggested that real acupuncture may have an effect on oxidative stress parameters in patients with FMS. Only a few research have examined the effectiveness of acupuncture in reducing oxidative stress. In a study conducted with rats with cerebral edema, decreased SOD activity increased with acupuncture and increased MDA levels decreased.[34] Acupuncture increases the level of endogenous opioids by activating pain-modulating pathways. Enkephalin, one of the endogenous opioids, has been shown to increase antioxidant capacity through the glutathione mechanism.[35] In our results, it can be thought that the alteration to the antioxidant side of glutathione levels may be related to the increase in endogenous opioid levels achieved by acupuncture. To the best of our knowledge, and in the light of a thorough literature review, our study represents the first effort to investigate the impact of acupuncture on oxidative balance, as measured by intracellular total GSH, GSH, and GSSG levels, in patients with FMS. The findings of this study may act as a foundation for future research in this field, as determining the effects of acupuncture on the oxidative stress level in FMS patients is thought to contribute to current treatment algorithms.

In the current study, there was a significant improvement in depressive symptoms, pain scores, disease and fatigue severity and QoL at the end of treatment both in the real and sham acupuncture groups, and this improvement was higher in the real acupuncture group than in the sham acupuncture group. There are conflicting data in the literature regarding the effect of acupuncture on depressive symptoms in FMS and there is no consensus.[27,36,37] Our results suggest that acupuncture may be effective on depressive symptoms in FMS. However, considering that BDI is a self-report scale, it is thought that future multidisciplinary studies are needed to support our findings. According to the literature, it is seen that real acupuncture is more effective in reducing pain intensity in patients with FMS than sham acupuncture. [27,38,39] In addition, our results are similar to the results of previous studies suggesting that acupuncture treatment in FMS is effective on functionality and disease severity assessed by FIQ scores.[27,36,37] Our study results support studies suggesting that acupuncture has a positive effect on QoL.[36-40]

Furthermore, there was no significant difference in the demographic characteristics, clinical parameters and oxidative stress levels of the patients in the real and sham acupuncture groups before treatment in our study. It is thought that the reliability of the results obtained from our study has increased due to the similar initial values of the two groups. Since oxidative stress mechanisms are thought to play a role in its pathophysiology, patients with FMS with a history of neurological, orthopedic, rheumatological and psychiatric diseases were not included in our study. In this way, it is thought that the role of oxidative stress mechanisms in the pathophysiology of FMS has been studied more clearly. In addition, the fact that the patients in our study did not receive any treatment for FMS increases the reliability in evaluating the effect of acupuncture on oxidative stress. As reported in the literature, randomization and blinding are important for the quality of the studies due to the placebo response observed with sham acupuncture.[26,27] The fact that our study is a single-blind, randomized study and the patients have not received acupuncture treatment before increases the reliability of our results. Another strength of our study is the technique used in the sham acupuncture group. In acupuncture studies, it is also preferred to place needles at sham points in control groups. However, needling at the sham points is thought to provide neuromodulatory inputs to the sensory nervous system. As a result, physiological changes that are indistinguishable from real acupuncture points can be seen with sham needling.[27] To prevent this situation, the same acupuncture points were used in the patients in the real and sham acupuncture group. In this way, a more reliable evaluation of the differences between real and sham acupuncture groups was provided.

The study's results on oxidative stress markers in FMS patients enhance our understanding of FMS pathophysiology, indicating the crucial role of oxidative stress. Our study demonstrates the relationship between acupuncture treatment and better oxidative balance, providing valuable information on potential treatments for FMS. These observations emphasize the importance of incorporating antioxidant strategies into FMS management, supporting a comprehensive

treatment approach. Future research should investigate the effectiveness of acupuncture in FMS treatments, which could influence clinical practice by including assessments of oxidative stress and personalized acupuncture procedures in treatment plans. This approach could greatly improve FMS treatment strategies by focusing on personalized medicine concepts.

Nonetheless, there are some limitations to this study. The first noticeable limitation is the low sample size. This situation is caused by the strict inclusion criteria into the study. Another limitation is that the sample was constituted with only female FMS patients. These results should be supported by studies to be conducted with patients with FMS of both sexes.

In conclusion, based on a comprehensive review of the existing literature, our study appears to be the first to evaluate oxidative stress levels through the measurement of intracellular GSH, GSH, and GSSG levels in patients with FMS in comparison to a control group. Our study findings suggest that measuring intracellular total GSH, GSH, and GSSG levels may serve as biomarkers for assessing oxidative stress among those with FMS. Furthermore, based on these findings, it may be inferred that authentic acupuncture may induce a clear alteration in the oxidative homeostasis among individuals with FMS. However, these results should be supported by studies to be conducted with larger samples in the future.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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