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Evaluation of oxidative stress in degenerative rotator cuff tears

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Background: Oxidative stress occurs as a result of the disruption of the balance between the formations of reactive oxygen species and antioxidant defense mechanisms during the conversion of nutrients into energy. Increased body oxidative stress has been reported to be involved in the etiology of several degenerative and chronic diseases. We hypothesized that the body oxidative stress level is higher in patients with atraumatic degenerative rotator cuff tear than that in healthy individuals.

Methods: The patients who underwent arthroscopic repair for atraumatic, degenerative rotator cuff tear were prospectively evaluated. A total of 30 patients (group 1, 19 females and 11 males; mean age: 57.33 ± 6.96 years; range: 50-77 years) and 30 healthy individuals (group 2, 18 females and 12 males; mean age: 56.77 ± 6 years; range: 51-72 years) were included in the study. The Constant and American Shoulder and Elbow Surgeons scoring systems were used to evaluate the clinical outcomes. Serum oxidative stress parameters of the patients and the control group were biochemically evaluated. Accordingly, thiol/disulfide (DS) balance (DS/native thiol [NT], DS/total thiol [TT]), Total Oxidant Status (TOS), oxidative stress index, and nuclear factor erythroid-2–associated factor-2 values were used as the biochemical parameters indicating an increase in the serum oxidative stress level. Total antioxidant status and NT/TT values served as the biochemical parameters indicating a decrease in the serum oxidative stress level.

Results: The study follow-up duration was 12 months. A statistically significant increase was observed in American Shoulder and Elbow Surgeons and Constant scores of patients who underwent arthroscopic rotator cuff repair relative to that during the preoperative period ($P = .01$). The values of biochemical parameters (DS/NT, DS/TT, TOS, oxidative stress index, and nuclear factor erythroid-2–associated factor-2), which indicated an increase in the serum oxidative stress, were significantly higher in preoperative patients than those in postoperative patients, albeit the control group values were significantly lower than those of the postoperative patients. The biochemical parameters (NT/TT and total antioxidant status) indicating a decrease in the serum oxidative stress levels were significantly higher in the postoperative patients than those in the preoperative patients and significantly lower than those in the control group.

The study protocol was approved by Harran University Ethics Committee (decision 19.02.10-28-31).

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Conclusion: High levels of markers indicating an increase in the serum oxidative stress in patients with degenerative rotator cuff rupture suggested that TOS may be involved in the etiopathogenesis of rotator cuff degeneration. Although the oxidative load decreases during the postoperative period, the fact that it is still higher than that in healthy individuals supports this claim.
Level of evidence: Level IV; Case Series; Prognosis Study
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Rotator cuff tear pathology is one of the most common shoulder pathologies.[20] There are two main factors in its etiopathogenesis: intrinsic and extrinsic. The main extrinsic factors are acromion type, subacromial spur, subacromial impingement, overuse of the shoulder, and trauma.[25] Several other factors such as genetic characteristics, age, hypervascularity, altered biology, diabetes, obesity, and metabolic and hormonal imbalance may also cause rotator cuff disease.[13,18] A few past studies have shown that body oxidative stress may increase in the pathogenesis of rotator cuff tears, like in the pathogenesis of other chronic and degenerative diseases.[21,38] Oxidative stress is defined as the disruption of the balance between the formation of reactive oxygen species and antioxidant defense mechanisms during the conversion of nutrients into energy.[22] Thiol groups, which are also called mercaptans and contain sulfhydryl (-SH) group, are reversibly converted to disulfides (DSs) containing covalent -S-S bonds in their structure, which play an important role in maintaining the oxidation and reduction balance between free oxidative radicals and antioxidant defense.[29] Nuclear factor erythroid-2- associated factor-2 (Nrf2) is a cellular transcription factor defined as the main coordination pathway that regulates the expression of genes responsible for encoding antioxidant and detoxifying proteins and provides cytoprotection. In the absence of any oxidative stress, Nrf2 is retained in the cytoplasm in an inactive position (INrf2). Under exposure to oxidative stress, Nrf2 is released and begins to accumulate in the cell nucleus. Active Nrf2 provides a transcription of cytoprotective and antioxidant proteins.[39] The total oxidative stress level in the serum, resulting from excessive free radical production and insufficient antioxidant mechanisms, is called the Total Oxidant Status (TOS).[9] Antioxidants undergo a synergistic interaction, and the total measurement of plasma levels is much more significant than the individual measurement. Therefore, the total antioxidant capacity is measured and is referred to as the total antioxidant status (TAS). To determine the oxidative stress balance clearly, the ratio of TOS to TAS was calculated, and the resulting value is called the total oxidative stress index (OSI) of the body.[9] In past studies, oxidative stress has been demonstrated to be effective in etiopathogenesis in the presence of inflammatory diseases, diabetes mellitus, cancer, neurodegenerative diseases, cardiovascular diseases, immune system disorders, and musculoskeletal system diseases.[6]

In this study, we examined the effect of body TOS as an etiological factor in atraumatic degenerative rotator cuff tears. We hypothesized that the body oxidative stress level is higher in patients with atraumatic degenerative rotator cuff tear relative to that in healthy individuals.

Patients and methods

This prospective study enrolled patients who applied to the Orthopedics and Traumatology outpatient clinic of Harran University Faculty of Medicine between November 2018 and June 2019 with the diagnosis of atraumatic full-thickness rotator cuff tear and did not respond to conservative treatment and accordingly underwent arthroscopic surgery. When selecting the patient group, patients who showed a rotator cuff tear by physical examination and radiological imaging and whose history of rotator cuff tear was not due to trauma were included in the study (group 1). Patients with massive, irreparable, Goutallier stage 4 tears, and biceps/labral pathologies were excluded from the study. Patients with a history of chronic or an additional disease such as hypertension, proteinuria, diabetes, renal failure, anemia, and a history of past surgery, alcohol use, and smoking were excluded from the study. Patients with pathology that could cause extrinsic impingement were not included in the study. In the control group, age-matched healthy individuals with the patients who did not have a history of smoking, alcohol, or any drug use were included in the study (group 2). Harran University Ethics Committee approval was obtained for the study protocol (decision no: 19.02.10-28-31). The written informed consent form was obtained from each individual included in the study. The study was conducted following the World Medical Association Declaration of Helsinki.

Medical history was taken from all patients who applied with pain and loss of movement in the shoulder region and who did not describe any history of trauma, and their systemic diseases were inquired. Detailed shoulder physical examinations were performed. Radiographic views were obtained from all patients, and additional pathologies were investigated. The diagnosis of full-thickness rotator cuff tear was confirmed, and surgery was decided based on magnetic resonance imaging (MRI) results. Demographic data of the patient and control groups are shown in Table I. The patients were evaluated clinically in their routine follow-up using the American Shoulder and Elbow Surgeons (ASES) score and Constant scoring systems (Table II). The values of both the scoring systems at preoperative and 1-year follow-up were included in the study. Group 1 was evaluated through the DeOrio and Cofield classification system based on the arthroscopic tear size and Goutallier classification based on the degree of fatty degeneration of the tear (Table II).[7,12,15] Patients with type 2 and type 3 acromion according to Bigliani classification, os acromiale, and acromial spur were not included in the study.[5]

Table I Demographic data of the patient and control groups

Variables	Group 1	Group 2	*P* value
Patients (60)	30	30	
Age (yr) (x ± s)	57.33 ± 6.96	56.77 ± 6	.7
Gender			
Female (%)	19 (63.3)	18 (60)	
Male (%)	11 (36.6)	12 (40)	

Student t-test. x: mean; s: standard deviation.

Serum oxidative stress markers of group 1 and group 2 were evaluated. Blood samples were collected from patients during the preoperative period and at the first year from controls and then compared with the blood serum samples of healthy individuals. As the serum oxidative stress parameters, native thiol/total thiol (NT/TT), DS/NT, DS/TT, TAS, TOS, OSI, and Nrf2 were evaluated. NT and TT are antioxidant defense compounds. While the ratio of NT/TT (%) increases under the dominance of the TAS antioxidative defense mechanism, it decreases in cases of oxidative stress (such as pathologies). While DS/NT (%), DS/TT (%), OSI, and Nrf2 increase under the conditions of increased oxidative stress, they decrease under the dominance of antioxidative defense.

Study of oxidative parameters

Blood samples collected from the patient group diagnosed with rotator cuff tear and the healthy control group after 8-12 hours of fasting were added to 8.5-mL gel biochemistry tubes. After keeping the blood samples at room temperature for 30 minutes, they were centrifuged at 3500 rpm for 10 min. The separated serum samples were transferred to sterile Eppendorf tubes and stored at −80°C. Biochemical analyses were performed according to the protocol suggested for the FineTest Sandwich ELISA (Enzyme-Linked ImmunoSorbent Assay) kit. Following the addition of 100 μL of serum samples to 96-well plates included in the kit, they were incubated at 37°C for 90 minutes. After incubation, the plate was emptied and washed twice with a washing solution and dried. Then, 100 μL of biotin-labeled antibody was added, and the solution was incubated at 37°C for 60 minutes. After incubation, the plate was emptied and washed thrice with a washing solution and then dried. Next, 100 μL of HRP (horse radish peroxidase)-streptavidin conjugate was added, and the solution was incubated at 37°C for 30 minutes. After incubation, the plate was emptied, washed five times with the washing solution, and dried. Next, 90 μL of the TMB (3,3'5,5'-Tetramethylbenzidine) substrate was incubated at 37°C in the dark for 20 minutes. After color formation was observed, 50 μL of the Stop Solution was added. Data were obtained through a microplate reader (Biotek-Cytation-1) Biotek-Cytation 1 (BioTek Instruments Inc., Winooski, VT, USA) at 450-nm absorbance (Nrf2).

The serum TAS level was measured by using the automated colorimetric method developed by Erel.[9] The dark blue 2.20-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical was reduced to the colorless 2.20-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) form and then determined. Calibration of the test was performed with Trolox, (Selleck Chemicals, Houston TX) a vitamin E analog, and referred to in mmol Trolox equiv/L. The serum TOS level was measured using a fully automated colorimetric method. The oxidation of ferrous ion o-dianisidine complex to ferric ion by oxidants in the sample was also measured. The density of the color associated with the number of oxidants in the sample was measured spectrophotometrically. Calibration was performed with hydrogen peroxide, and the results were obtained as micromolar hydrogen peroxide equivalents per liter (μmol H_2O_2 Equiv/L). The OSI, an indicator of oxidative stress, was calculated according to the following formula after measuring the TOS and TAS levels: OSI (arbitrary units) = [(TOS, μmol H_2O_2 Equiv/L)/(TAS, μmol Trolox equiv/L) ×100].

NT and TT were studied with the automatic colorimetric measurement method by Erel and Neselioglu.[8] According to this method, first, dynamic DS bonds (S–S–) in the serum were reduced to functional thiol groups (-SH) with NaBH4 (sodium borohydride). Then, the unused NaBH4 was removed with formaldehyde. Thereby, extra reduction of 5,5′-dithiobis-2 nitrobenzoic acid and DS bridges was prevented. The serum TT amount was measured with a modified Ellman reagent. A DS level of 30 was obtained by subtracting the NT from the TT and dividing the difference by two [DS = (TT-NT)/2]. Then, the percentage ratios of DS/NT, DS/TT, and NT/TT were calculated. As albumin is the main source of serum thiols, the corrected NT, TT, and DS levels for albumin were calculated according to the corrected TT = TT (μmol/L)/albumin (g/dL), corrected NT = NT (μmol/L)/albumin (g/dL), and corrected DS = DS (μmol/L)/albumin (g/dL) formulas.[3]

All the patients were diagnosed with the same MRI device and operated in the same operating room by the same surgeon (BS). Since anesthetic drugs may affect stress oxidase enzymes, fasting blood samples were collected in the morning before anesthesia. In order to minimize the stress factor, all surgeries were considered as the first case. Interscalene block combined with anesthesia was applied to patients together with general anesthesia to reduce the postoperative pain. All patients underwent arthroscopic surgery and were operated on in the lateral decubitus position. The tear size and shape were determined by measuring the widest opening after 1- to 2-mm débridement of the rotator cuff tear. The width of the probe tip (6 mm) was used for the measurement. Double-row repair was performed for all patients. Acromioplasty was applied to patients with advanced degeneration of the coracoacromial ligament.

Velpeau bandage was applied to patients on the operating table. Bandage application continued for up to 4 weeks postoperatively. All patients were prescribed home exercises, and the exercises were followed up with controls. Shoulder pendular exercises were started from the first postoperative day (0-15 days). Active assisted exercises were started at the end of the second week. For the first 6 weeks, overhead movements were avoided. Active exercises were started in the fourth week (1-3 months). Return to sports was allowed beginning from the sixth month.

Statistical analysis

The conformity of the data to the normal distribution was tested through the Shapiro–Wilk test, and Student t (for normally distributed variables) and Mann–Whitney U-tests (for non-normally distributed variables) were used for the comparison of the numerical variables in two independent groups. In addition,

Table II Scoring and classification values of the patient and control groups

	Group 1		*P* value
	Preoperative	Postoperative	
DeOrio and Cofield classification			
Small	5 (16.6%)		
Medium	10 (33.3%)		
Large	15 (50%)		
Goutallier classification			
Grade 0	4 (13.3%)		
Grade 1	12 (40%)		
Grade 2	8 (26.6%)		
Grade 3	6 (20%)		
ASES score	15.78 ± 8.93	79.45 ± 17.1	**.001***
Constant score	22.77 ± 8.61	78.87 ± 17.7	**.001***

ASES, American Shoulder and Elbow Surgeons.
* The *P* value with the significance level .05; Wilcoxon test.

paired t (for normally distributed variables) and Wilcoxon tests (for non-normally distributed variables) were employed to compare numerical variables at two different time points. Kruskal–Wallis and Dunn multiple comparison tests were performed for comparison of the numerical measurements between more than two categories. Relationships between numerical measurements were tested with the Spearman correlation coefficient. SPSS for Windows 24 program (IBM, Armonk, NY, USA) was used in the analysis, and $P < .05$ was considered to indicate statistical significance. Student t (for normally distributed variables) and Mann–Whitney U-tests (for non-normally distributed variables) were applied for male–female comparisons in two independent groups.

Results

No patients were lost during the follow-up period. The demographic characteristics of the patients are shown in Table I.

There were supraspinatus and infraspinatus tears in 16 patients, isolated supraspinatus tears in 13 patients, and subscapularis and supraspinatus tears in 1 patient. The follow-up period for all patients was 1 year. The ASES and Constant scoring results of the patients are shown in Table II, which are in line with the DeOrio-Cofield classification and Goutallier classification. Postoperative ASES and Constant scores were statistically significantly higher than the corresponding preoperative scores ($P = .001$).

No statistically significant relationship was noted between DeOrio and Cofield and Goutallier classification and the preoperative ASES and Constant scores ($P = .56$ and $P = .44$; $P = .73$ and $P = .84$, respectively). There was no statistically significant relationship between postoperative ASES and Constant values with DeOrio and Cofield and Goutallier classifications ($P = .1$ and $P = .89$; $P = .24$ and $P = .74$; Table III).

Preoperative ASES and Constant scores and DeOrio and Goutallier classifications did not show a statistically significant relationship with both preoperative and postoperative oxidative stress parameters (Table IV). There was no significant difference in terms of gender in the preoperative and postoperative oxidative stress values between the patient and control groups ($P > .05$).

Postoperative mean NT/TT values were statistically significantly higher than the corresponding preoperative values ($P = .001$). The mean NT/TT ratio in group 2 was statistically significantly higher than both preoperative and postoperative values in group 1. The mean values of TAS in group 2 and that in the postoperative period were statistically significantly higher than the values in the preoperative period. The mean TAS values in group 2 were higher than those in the postoperative values in group 1, but not statistically significant ($P = .487$). Postoperative oxidant oxidative stress parameters (DS/NT, DS/TT, TOS, and OSI) and the mean Nrf2 values were statistically significantly lower than the outcomes of preoperative measurements. Group 2 DS/NT, DS/TT, TOS, and OSI mean values were statistically significantly lower than both the preoperative and postoperative mean values in group 1 (Table V).

Discussion

The notion that increased body oxidative stress may be a factor in the etiopathogenesis of degenerative rotator cuff tear encouraged the design of this study. We compared the systemic oxidative stress parameters, dynamic thiol-DS balance, and the Nrf2 levels between patients diagnosed with degenerative full-thickness rotator cuff tear and an age-matched healthy control group. In addition, we compared the preoperative and postoperative values of these parameters in the patients. We noted that all these biochemical parameters showed a tendency of increasing oxidative stress in the patient group. A satisfactory improvement in clinical scores was detected in the postoperative period. We observed that the oxidative load decreased in the postoperative period with clinical improvement, but was still higher than that in healthy individuals. We thus showed that increased oxidative stress may be associated with rotator cuff degeneration.

Oxidative stress has been reported to trigger the etiopathogenesis of several diseases such as inflammatory diseases, diabetes mellitus, aging, cancer, neurodegenerative diseases, cardiovascular diseases, and immune system disorders.[4,27,31] Diseases such as developmental dysplasia of the hip, pes equinovarus, and osteoarthritis in the musculoskeletal system have been reported to be associated with oxidative stress.[1,2] The effect of oxidative stress on tendinopathies has been demonstrated in Achilles and patellar tendons in rat and human studies.[10,16] In fact, Nho et al reported that oxidative stress may trigger shoulder rotator cuff tendon injury.[26]

Table III The relationship between the degree and fatty degeneration of the tear and clinical scores

Clinical classifications	Preoperative ASES	Preoperative Constant	Postoperative ASES	Postoperative Constant
DeOrio				
Small (n = 5)	14.52 ± 5.28	25.6 ± 7.64	77.96 ± 20.92	71 ± 22
Goutallier				
Medium (n = 10)	14.65 ± 9.96	17.7 ± 5.72	74.45 ± 17.41	76 ± 16.69
Large (n = 15)	16.96 ± 9.52	25.2 ± 9.39	83.29 ± 15.64	83.4 ± 16.85
P value	.56	.10	.44	.24
Grade 0 (n = 4)	18.15 ± 6.17	23.5 ± 6.95	74.53 ± 21.81	70.25 ± 25.72
Grade 1 (n = 12)	14.15 ± 9.11	21.5 ± 7.59	79.68 ± 14.77	80.83 ± 12.81
Grade 2 (n = 8)	18.31 ± 8.81	22.75 ± 11.49	77.88 ± 21.02	75.75 ± 22.2
Grade 3 (n = 6)	14.1 ± 11.14	24.83 ± 8.93	84.4 ± 15.5	84.83 ± 15.79
P value	.73	.89	.84	.74

ASES, American Shoulder and Elbow Surgeons.
Kruskal–Wallis test.

Table IV The relationship between ASES, Constant, DeOrio, and Goutallier findings and oxidative stress parameters

Oxidative stress measurements	Preoperative ASES	Preoperative Constant	Preoperative DeOrio-Cofield	Preoperative Goutallier
	r	r	r	r
Preoperative oxidative stress measurements				
DS/NT	−0.009	0.176	−0.256	−0.006
DS/TT	−0.009	0.176	−0.256	−0.006
TOS	−0.065	0.002	0.049	0.005
OSI	0.026	0.217	0.280	0.250
Nrf2†	−0.249	−0.336	0.120	0.000
NT/TT†	0.003	0.176	0.253	0.004
TAS†	−0.082	−0.160	−0.289	−0.371*
Postoperative oxidative stress measurements				
DS/NT	−0.058	−0.068	−0.052	−0.054
DS/TT	−0.058	−0.068	−0.052	−0.054
TOS	0.177	0.233	0.230	−0.113
OSI	0.071	0.136	−0.049	−0.163
Nrf2†	−0.265	−0.133	−0.097	−0.055
NT/TT†	0.058	0.068	0.052	0.054
TAS†	0.037	0.031	0.211	0.033

DS, disulfide; *NT*, native thiol; *TT*, total thiol; *TAS*, total antioxidant status; *TOS*, total oxidant status; *OSI*, oxidative stress index; *Nrf2*, nuclear factor erythroid 2–related factor 2.
The minus sign indicates negative correlation; the plus sign indicates positive correlation.
* r: Spearman rank correlation coefficient. The significance level of 0.05. Weak correlation (0.2-0.4), moderate correlation (0.4-0.6), strong correlation (0.6-0.8), and very strong correlation (>0.8).
† Antioxidant defense parameters.

Thiol, which is one of the molecules that prevent oxidative stress, is converted to DS (-S-S-) by oxidation, which acts as an antioxidant and prevents oxidative stress. This action is reversible and crucial in maintaining antioxidant defense.[28] It has been shown that plasma thiol/DS balance is influenced in patients who undergo dialysis and in patients with chronic kidney disease, diabetes mellitus, cardiovascular diseases, malignancies, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease, multiple sclerosis, liver diseases, inflammation, and osteoarthritis.[16,33] In this study, we investigated the relationship between thiol-DS balance and atraumatic rotator cuff tear. We also studied the thiol-DS balance parameters as the antioxidant group NT/TT and oxidant group DS/NT and DS/TT. While the antioxidative defense mechanism is dominant in healthy people, oxidative stress parameters are expected to increase during the disease process. In our study, while the mean values of the antioxidant NT/TT ratio in preoperative patients were statistically significantly lower than those in postoperative patients and healthy individuals, the mean values of the DS/NT ratio and DS/TT ratio were statistically significantly higher. The decrease in the NT/TT ratio,

Table V Analysis of oxidative stress parameters by groups

Oxidative parameters	Group 1		Group 2	*P* value		
	Preoperative	Postoperative	Control	P_1	P_2	P_3
DS/NT (%)	10.17 ± 3.37	5.91 ± 2.08	4.51 ± 2.13	.001*	.001*	.016*
DS/TT (%)	8.33 ± 2.21	5.22 ± 1.64	4.07 ± 1.75	.001*	.001*	.011*
TOS	14.26 ± 2.03	12.55 ± 2.00	10.84 ± 1.26	.004*	.001*	.001*
OSI (AU)	2.1 ± 3.12	1.54 ± 2.15	1.02 ± 0.33	.001*	.001*	.014*
Nrf2†	4.99 ± 0.64	3.98 ± 0.24	3.77 ± 0.48	.001*	.001*	.033*
NT/TT (%)†	83.33 ± 4.42	89.55 ± 3.27	91.85 ± 3.51	.001*	.001*	.011*
TAS†	0.92 ± 0.21	1.09 ± 0.27	1.15 ± 0.29	.001*	.001*	.487

DS, disulfide; NT, native thiol; *TT*, total thiol; *TOS*, total oxidant status; *OSI*, oxidative stress index; *AU*, arbitrary units; *Nrf2*, nuclear factor erythroid 2–related factor 2; *TAS*, total antioxidant status.

* The *P* value with the significance level .05; in-group comparisons, Wilcoxon test; comparisons between groups Mann–Whitney u test. Median [25%-75%]. P_1, the *P* value of preop and postop group comparison; P_2, the *P* value of preop and control group comparison; P_3, the *P* value of postop and control group comparison.

† Antioxidant defense parameters.

which indicates the increase in the oxidative stress level, and the increase in the DS/NT and DS/TT ratios were prominent in the patient group. Our results suggest that free oxygen radicals can disrupt the dynamic thiol-DS balance, thus creating oxidative stress.

Considering the mean NT/TT values, we noted that the mean TAS values were statistically significantly higher in the healthy group than those in the patient group. Similarly, postoperative mean TAS values were statistically higher than the preoperative values. Increases in the TAS levels were noted in the postoperative period. Neelofar et al report that total antioxidants were consumed during the increase of oxidative stress and that total antioxidants are regenerated during the healing process of the disease.[24] The present results are in line with those of the study by Neelofar et al. The postoperative increase in TAS supports the success of arthroscopic surgery. Increases in the TOS values were statistically significantly higher in the preoperative patient group than those in the postoperative period and healthy individuals. This significant increase in the TOS values is in line with the increase in the systemic oxidative stress values in rotator cuff tears. Thus, the increase in the OSI is also a parameter that indicates a disturbance in the balance in the oxidative direction. In our study, we found this index at the highest level in the patient group and the lowest level in healthy individuals.

Nrf2 has been reported to exert protective effects on the oxidative system in several chronic and degenerative diseases.[14] Sun et al reported an increase in Nrf2 and a concomitant improvement in patellar tendon stem cells as well as a subsequent decrease in Nrf2 with recovery when they exposed rat patellar tendon stem cells to oxidative stress with hydrogen peroxide *in vitro*.[32] In our study, the Nrf2 values were statistically significantly higher in preoperative patients than those in postoperative patients, while the lowest Nrf2 levels were detected in healthy individuals. Meng et al reported that Nrf2 increased in the presence of rupture in rat Achilles tendons and decreased during the recovery period.[19] Nrf2 is an antioxidant defense mechanism molecule, which may explain its significantly higher levels in group 1 than in group 2. Tohidnezhad isolated tenocytes from the rat Achilles tendon and exposed it to oxidative stress, reporting that the tendon healed through Nrf2.[34] These findings by Sun and Tohidnezhad are in parallel with our results. To the best of our knowledge, the only study in the literature that directly demonstrates the effect of Nrf2 on oxidative stress in human rotator cuff tendon tears is Gallorini's study published in 2020 on cell cultures. Gallorini stated that rotator cuff tears induced by oxidative stress can be prevented by antioxidant enzymes provided by Nrf2 expression.[11] Notably, our study seems significant considering that Nrf2 was studied in a human model.

In our study, the markers of an increase in oxidative stress included the increase in TOS, OSI, DS/NT, DS/TT, and Nrf2 parameters, while those indicating a decrease in the oxidative stress were the increase in TAS and NT/TT parameters. We evaluated our patients in terms of clinical and oxidative biochemical parameters and found that preoperative oxidative stress was high and that the parameters indicating oxidative stress load had decreased after arthroscopic surgery. We achieved satisfactory results in clinical ASES and Constant score values in the patients. In our study, we saw that the pain of our patients completely disappeared or decreased. It was shown that pain had an effect on increasing oxidative stress. It was reported that there was a decrease in oxidative stress with the decrease or disappearance of pain.[23,30] Similarly, in our study, it was observed that oxidative stress decreased with improvement in pain complaints and clinical scores. Together with the clinical improvement in patients, a decrease was noted in the oxidative load, which indicated that the treatment was effective. However, the parameters suggesting the oxidative load relative to those in healthy subjects were at higher

levels, although not as much as that during the preoperative period. These findings support that increased oxidative stress may be a risk factor in the pathogenesis of the rotator cuff. Although there was a decrease in the oxidative load during the postoperative period, the oxidative load at high levels when compared to that in the healthy control group may pose a risk of recurrence for patients in the future. Yoshida et al, in support of our results, demonstrated that the risk of rotator cuff rupture increased with increased oxidative stress.[37] Our study thus stresses the importance of oxidative stress enzymes. Wu et al stated that rotator cuff tendons torn by degeneration healed poorly after repair.[36] The increase in oxidative stress may thus be a risk factor for the formation of rotator cuff tears as well as a risk factor that affects the healing process.

The mean age of our study subjects was 57.33 ± 6.96 years in the patient group and 56.77 ± 6 years in the control group ($P = .7$). Touheti and Itoi stated that the possibility of degenerative tendinopathic changes in the rotator cuff increased after the third decade.[35] In order to minimize the effect of age on oxidative stress, we included patients of a similar age group and healthy people. To restrict the impact of confounding variables on the analysis, the operations were performed in the same operating room and by the same surgeon and the same anesthesia team. Patients with degenerative rotator cuff tears without chronic disease were selected considering that chronic diseases and systemic disorders also increase oxidative stress.[6] Smokers were excluded from the study because smoking increases oxidative stress in the rotator cuff tendons.[17]

Limitations

The study has some limitations. The follow-up period of the patients was 12 months, which may be a short period for clinical follow-up of degenerative rotator cuff tears. Another limitation is related to the evaluation of rotator cuff healing clinically, but not radiologically (MRI). The oxidative stress data were obtained from the blood of patients and healthy individuals. Since the rotator cuff tissue samples were not collected from individuals, the oxidative stress levels were not examined at the tissue level.

Conclusion

Clinically satisfactory outcomes can be obtained after arthroscopic repair in patients with degenerative rotator cuff tears. High levels of markers (DS/NT, DS/TT, TOS, OSI, and Nrf2) act as an indication of increased serum oxidative stress in patients with degenerative rotator cuff rupture, suggesting that the total oxidative stress may also be a factor in the etiopathogenesis of rotator cuff degeneration. Although the oxidative load decreased in the postoperative period, it was still higher than that of healthy individuals, which supports our notion. The decrease in the oxidative markers and increase in antioxidant markers (NT/TT and TAS) after arthroscopic treatment suggest that rotator cuff pathologies may be a cause of oxidative stress.

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