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Research Article

Evaluation of oxidative stress in gout disease; thiol-disulfide homeostasis and ischemia-modified albumin levels

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Abstract

Objectives: Gout is a common and easily treated disease characterized by the accumulation of monosodium urate crystals in both joints and other tissues. Monosodium urate crystals are the main stimulants for initiating and maintaining an inflammatory response. Oxidative stress is also an early change in gout pathogenesis together with inflammation. This study aimed to investigate the presence of oxidative stress in gout together with thiol–disulfide homeostasis and ischemia-modified albumin (IMA) levels. We aimed to compare these parameters with inflammation marker C-reactive protein (CRP) and high-sensitivity C-reactive protein (hsCRP).

Methods: Levels of native thiol, total thiol, disulfide, IMA, CRP, and hsCRP were detected in patients with gout (n=50) and healthy subjects (n=50). Student's t-test and the Mann-Whitney U test were used for istatistical analysis.

Results: Native thiol, total thiol, and index 3 (native thiol/total thiol×100) were significantly lower in the patient group, while disulfide, index 1 (disulfide/native thiol×100), index 2 disulfide/total thiol×100), IMA, CRP, and hsCRP were significantly higher. In addition, elevation in native thiol, total thiol, and disulfide levels was detected as disease duration increased.

Conclusion: The present study has shown the role of oxidant damage in gout disease. Additional studies are needed to identify sources of oxidative stress in gout.

Keywords: C-reactive protein, dynamic thiol-disulfide homeostasis, gout, high sensitive C-reactive protein, ischemia-modified albumin.

Gout is a common and easily treated disease characterized by the accumulation of monosodium urate (MSU) crystals in both joints and other tissues. Increased serum urate concentration is the major risk factor for gout. Population-based studies conducted in Asia, Europe, and North America have shown that the incidence interval is between 0.6 and 2.9 among 1000 people over 1 year, and the prevalence in the adult population was reported to be between 0.68% and 3.90% [1].

Uric acid is a pro- and antioxidant, a neurostimulant, inflammation-inducing, and an immune response-evoking biologically active molecule. Hyperuricemia is closely related to gout epidemiologically, as it is a major predictive factor for disease progression [2]. High extracellular sodium concentration and physiological pH account for approximately 98% of MSU. Acute attacks of arthritis occur when the serum urate concentration usually exceeds 6.8 mg/dL (408 µmol/L). Cation concentration, temperature, viscosity, and hydrogen ion formation affect the formation of MSU crystals in the synovial fluid [3]. MSU crystals are the main stimulants for initiating an inflammatory process. MSU crystals are phagocytosed as for-

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eign particles. Phagocytosis causes the release of interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), monocyte chemotactic protein-1 (MCP-1), and CC chemokine receptor 7, as well as prostaglandins and adhesion molecules [4, 5].

One of the early changes seen in gout with these inflammatory processes is the presence of oxidative stress. This causes the accumulation of reactive oxygen species (ROS) and the production of proinflammatory stockings [6]. This situation is also known to cause degeneration in the articular tissues [7]. Thiol is an organic compound having sulfhydryl groups to compensate for oxidative stress. Protection of cells from oxidative damage is mostly done by low-molecular weight thiol and sulfhydryl stocks. Thiol (-SH) serum levels are correlated with antioxidant status, while disulfide (S-S) levels are correlated with exposure to oxidation [8].

Free radicals alter the N terminal region of albumin. This altered form is called ischemia-modified albumin (IMA) [9]. IMA levels also give valuable opinions about ROS exposure. C-reactive protein (CRP) synthesized by the hepatocytes is an acute-phase protein. Elevated CRP levels are known to be supporting the diagnosis of various diseases. High-sensitivity C-reactive protein (hsCRP) is more sensitive than standard CRP, and it gives a better measure of inflammation [10]. Gout is also an inflammatory disease. The relationship between oxidative stress and the presence of inflammation is well known. CRP and hsCRP, which are frequently used as inflammation markers in clinical laboratories, were evaluated together with the mentioned oxidative stress markers. For all these reasons, we aimed to evaluate the inflammatory status and oxidative stress with these biomarkers in patients with gout.

Materials and Methods

Patient population

Gout patients meeting the criteria of the American College of Rheumatology were included in the study [11]. The control group was selected from healthy relatives of patients who did not have any chronic diseases. A total of 100 volunteers, 50 patients and 50 controls, were included in the study. Participants were selected from individuals between the ages of 18 and 90 years. Individuals who smoked or consumed alcohol were excluded from the study. The study was approved by the local ethics committee (approval number: E2-21-402), and written consent was obtained from all participants according to Helsinki Criteria.

Analysis

Venous blood samples after 12 h of fasting were collected from all participants. The tubes were centrifuged at 3500 rpm for 10 min to serum. Separated sera were aliquoted into Eppendorf tubes and stored at -80°C until the time of analysis. IMA [12] and thiol–disulfide homeostasis tests (native thiol, total thiol, and disulfide) [13] were measured spectrophotometrically, while CRP and hsCRP levels were detected with the Beckman Coulter AU680 device by the immunoturbidimetric method. The indices of thiol-disulfide homeostasis were also calculated and recorded (index 1: disulfide/native thiol×100, index 2: disulfide/total thiol×100, index 3: native thiol/total thiol×100).

Table 1. Characteristics of patients with gout

Feature	All patients (n=50)	
	n	%
Age, mean±SD (years)	55.10±15.72	
Disease duration, median (min-max) (years)	3 (0–20)	
Gender		
Female	9	18
Male	41	82
Clinic		
Having an attack	49	98
Number of attacks, median (min-max)	2 (0–8)	
Hypertension	28	56
Diabetes mellitus	15	30
Atherosclerotic coronary heart disease	11	22
Tophus	5	10
Drug use		
None	8	16
Colchicine	10	20
Uricolysis	12	24
Colchicine+uricolysis	14	28
Colchicine+uricolysis+steroid	3	6
Colchicine+steroid	2	4
Colchicine+adenuric	1	2
Laboratory, median (min-max)		
Erythrocyte sedimentation rate (mm/h)	19 (2-65)	
Uric acid (mg/dL)	7.5 (4.2-13.60)	
White blood cell (10^3 cells/μL)	8.95 (4.5-19)	
Hemoglobin (g/dL)	13.84±1.46	
Platelet (10^3 cells/μL)	238.5 (134-593)	
Creatinine (mg/dL)	1.0 (0.6-1.7)	
Alanine aminotransferase (U/L)	32 (9-135)	
Gamma-glutamyl transferase (U/L)	44 (12-180)	
Fasting blood glucose (mg/dL)	102 (76-287)	
Triglyceride (mg/dL)	159 (55-493)	
Total cholesterol (mg/dL)	178 (128-278)	
High-density lipoprotein (mg/dL)	44 (30-74)	
Low-density lipoprotein (mg/dL)	122±27.82	

Statistical analysis

All statistical analyses were carried out with the IBM SPSS Statistic v.22 programs. Normality tests were done with the Shapiro-Wilk test. Results were presented as mean±SD for parametric variables, and median (min-max) for nonparamet-

Table 2. Serum native thiol, total thiol, disulfide, index 1, index 2, index 3, IMA, CRP, and hsCRP levels (mean +/- SD) in the patients and control groups

Parameters	Patients with gout (n=50)	Control (n=50)	p
Native thiol (µmol/L)	218.85±81.65	406.50±43.73	<0.001*
Total thiol (µmol/L)	251.33±88.26	438.23±44.06	<0.001*
Disulfide (µmol/L)	16.24±4.43	15.86±2.11	0.586
Index 1	7.60 (4.86-21.03)	3.95±0.68	<0.001*
Index 2	6.59 (4.43-14.80)	3.65±0.59	<0.001*
Index 3	86.82 (70.39-91.14)	92.70±1.18	<0.001*
IMA (ABSU)	0.919±0.105	0.859±0.074	0.001*
CRP (mg/L)	8.6 (0.7-210.9)	2.3 (0.3-21.2)	<0.001*
hsCRP (mg/L)	7.25 (0.5-160.5)	1.85 (0.1-21.5)	<0.001*

P<0.05 was considered statistically significant. P values with an asterisk indicate differences between the parameters. Index 1: Disulfide/native thiol×100; Index 2: Disulfide/total thiol×100; Index 3: Native thiol/total thiol×100; IMA: Ischemia-modified albumin; CRP: C-reactive protein; hsCRP: High-sensitivity C-reactive protein.

ric variables. Student's t-test and the Mann-Whitney U test were used for the analysis of numerical variables. Correlation analyses were performed using Pearson's and Spearman's tests. Receiver operating characteristic (ROC) analysis was carried out for parameters showing significant differences between patient and control groups. P<0.05 was accepted as a level of statistical significance for all tests.

Results

Of the total patients, 9 females and 41 males (age mean±SD =55.10±15.72) in the patient group and 14 females and 36 males in the control group (age mean±SD =52.28±14.94) were analyzed. The demographic and clinical features of the patient group are summarized in Table 1.

Native thiol, total thiol, and index 3 were significantly lower in the patient group, while disulfide, index 1, index 2, IMA, CRP, and hsCRP were significantly higher. Disulfide levels were also increased in gout patients but the change was not statistically significant (Table 2). On the other hand, the measured parameters were compared between those who received drug therapy (n=42) and those who did not (n=8). Accordingly, there was no significant difference between the patients who received and did not receive drug therapy for native thiol, total thiol, disulfide, IMA, CRP, and hsCRP (p values were 0.334, 0.328, 0.337, 0.313, 0.169, and 0.169, respectively).

Native thiol, total thiol, and disulfide levels were correlated with prolonged disease duration (p values were 0.032, 0.028, and 0.028, respectively). ROC analysis was done to detect parameters with the best power of discriminating between patients and healthy subjects (Fig. 1). Accordingly, native thiol, total thiol, index 1, index 2, and index 3 provided good discriminating power among the analyzed parameters with the specified cutoff values (AUC were 0.982, 9.975, 0.999, 0.900, and 0.999, respectively). As a result of the correlation analysis, CRP and hsCRP showed negative correlations with native thiol, total thiol, disulfide, and index 3 (p values for CRP were <0.001, <0.001, 0.014, and <0.001, respectively; p values for hsCRP were <0.001, <0.001, 0.010, and <0.001, respectively). They showed a positive correlation with index 1, index 2, and IMA (p<0.001 for all) (Fig. 2).

Discussion

In addition, CRP and hsCRP levels were evaluated together in terms of inflammation in these patients. Our results revealed that native thiol, total thiol, and index 3 are significantly lowered in gout patients while, index 1, index 2, IMA, CRP, and hsCRP are significantly elevated (Table 2). Native thiol, total thiol, and disulfide levels increased significantly as the disease duration increased. It was observed that the measured inflammation markers and the parameters of oxidative stress were in a significant correlation. ROC analysis has shown that native thiol, total thiol, index 1, index 2, and index 3 parameters have quite high sensitivity and specificity in the discrimination of patients from controls at determined cutoff levels. These results have directed us toward the close relationship between oxidative stress (measured by thiol-disulfide homeostasis and IMA) and gout disease, and it also correlates with the severity of inflammation. The present study is the first report to evaluate oxidative stress by means of thiol-disulfide homeostasis and IMA in gout patients. Our data are consistent with previous oxidative stress studies in gout [14, 15].

Oxidative stress is expressed as the imbalance between ROS production and antioxidant mechanisms [16]. Thiols are among the major constituents of serum antioxidants, and they have critical roles in ROS defense. Native thiols are the -SH groups found free of glutathione conjugated and reduced in and out of cells. Native thiols (-SH) can be oxidized and converted to disulfide (-S-S-) groups. This is a dynamic and reversible process. Oxidized (-S-S-) and reduced (-SH) thiol forms together compose the total thiols [13]. The amino acids aspartyl-alanyl and histidine-lysine located in the N-terminal region of albumin bind nucleic acids, metals such as nickel, copper and cobalt. Free radicals formed during ischemia and reperfusion, acidosis, and improper functioning of the sodi-

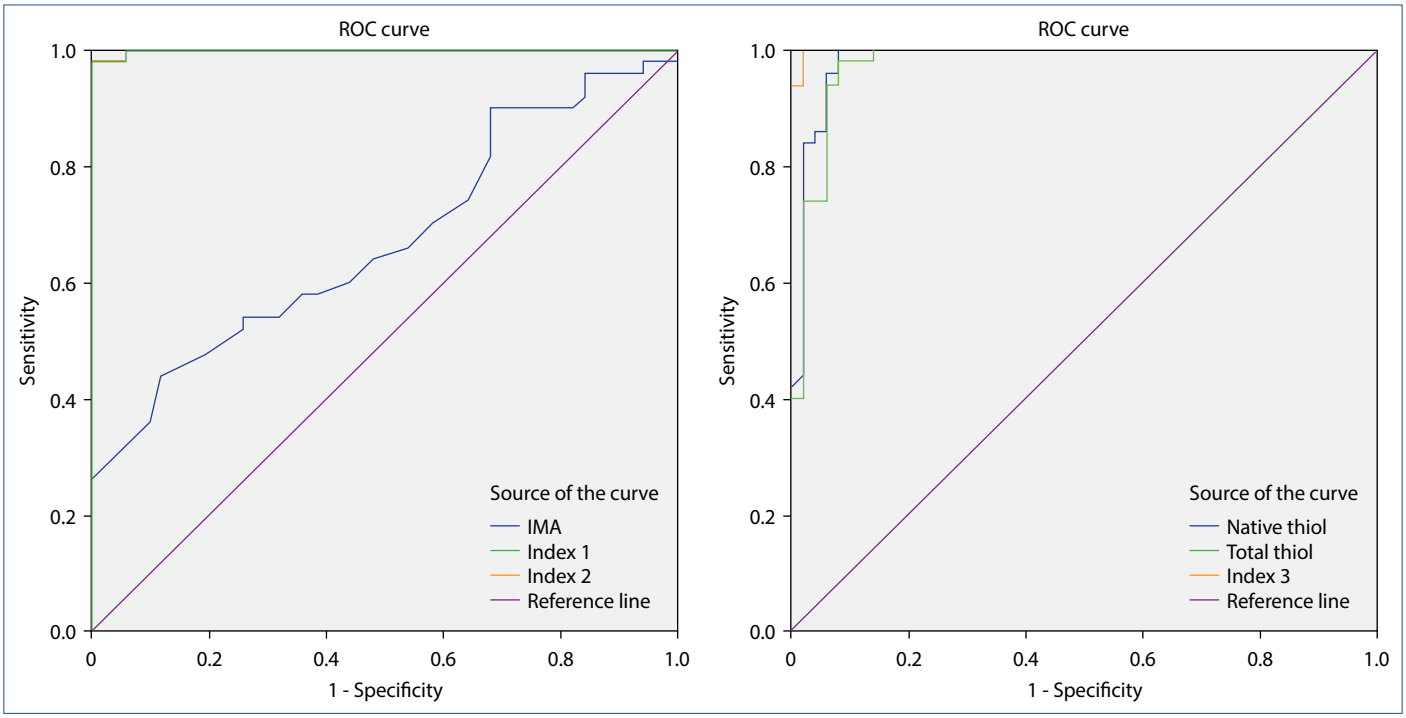

Figure 1. ROC analysis results.

ROC: Receiver operating characteristic; IMA: Ischemia-modified albumin; Index 1: Disulfide/native thiol×100; Index 2: Disulfide/total thiol×100; Index 3: Native thiol/total thiol×100.

um-potassium pump are the modifications affecting the N-terminal region. Free radicals alter the N-terminal region of albumin, and it cannot bind metal ions anymore it is called IMA. Normally, IMA is a total of 1%-2% of total albumin. But under ischemic conditions, its level increases to 6%-8%, which makes IMA a useful marker of oxidative stress [17, 18]. For these reasons, in the presence of increased oxidative stress, it is expected that native thiols will decrease by oxidation, and disulfide levels will increase relatively. In addition, a significant increase in IMA levels will be expected.

The formation of hyperuricemia increases the production of free oxygen radicals, supports lipid peroxidation, and increases the expression of proinflammatory factor [14]. During the development of gouty arthritis, MSU enters cells via endocytosis and induces inflammation [15]. MSU stimulates synovial cells, monocyte macrophages, and neutrophils to produce IL-1β, which promotes the release of a number of inflammatory cytokines such as IL-6, TNF-α, and MCP-1, leading to the spread of inflammation [19]. Additionally, sustained oxidative stress can lead to chronic inflammation [20]. Overproduction of ROS is a pathogenetic factor in acute gouty arthritis [21]. Excessive production of ROS activates inflammation, particularly the protein 3 containing NACHT, LRR, and PYD domains and promotes IL-1β production in gouty arthritis [22]. MSUs undergo phagocytosis of neutrophils. The resulting phagolysosomes are destabilized by ROS, cytokine release, proteases, arachidonic acid products, prostaglandins, and myeloperoxidase. This triggers membrane disruption and cell death [23]. In synovial cells exposed to MSU crystals and IFN-γ, NO- is produced by inducible nitric oxide synthase enzyme [24]. Synoviocytes form a part of the inborn immune response as they trigger an inflammatory process by the production of electronically unstable molecules, such as nitric oxide (NO-), O_2-, OH-, alkoxide (RO-), ROO-, and H_2O_2, hypochlorous acid, ozone (O_3), and singlet oxygen [25, 26]. Studies are reporting that these crystals attract immunoglobulins, lipids, and complement factors to themselves, which in turn causes the formation of immune complexes in various cells. According to this information, it is seen that ROS and inflammation increase together in the presence of gouty arthritis and play an active role in pathogenesis. The data of this study are in agreement with the presented information.

A recent study demonstrated that celery seed extract treatment decreased serum levels of uric acid and xanthine oxidase and ROS and increased serum levels of superoxide dismutase and glutathione peroxidase in mice with hyperuricemia. It has also been claimed that in rats with acute gouty arthritis, celery seed extracts may have antigout properties, in part through anti-inflammatory and antioxidative effects, by relieving swelling of the ankle joints and reducing inflammatory cell infiltration [14]. Another study on rats with gouty arthritis showed that administration of MSU caused significant increases in oxidative stress parameters (malondialdehyde and total oxidant status). In addition, significant decreases in antioxidant defense systems (glutathione, superoxide dismutase, and total antioxidant status) were observed [27]. In a study they carried out on birds with gout, Chakravarthi et al. [28] showed that antigout treatment reduced uric acid and xanthine oxidase levels and

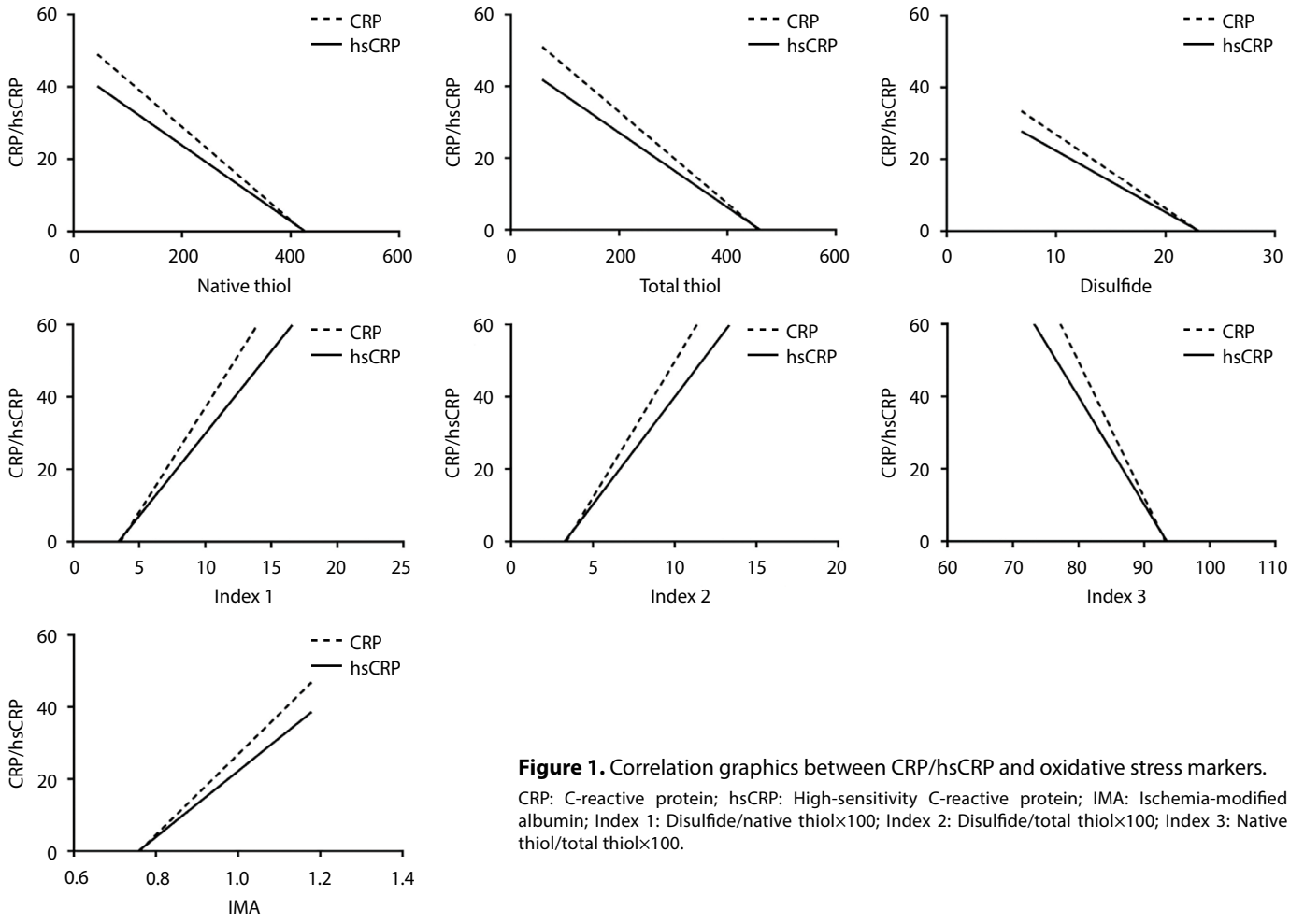

Figure 1. Correlation graphics between CRP/hsCRP and oxidative stress markers.

CRP: C-reactive protein; hsCRP: High-sensitivity C-reactive protein; IMA: Ischemia-modified albumin; Index 1: Disulfide/native thiol×100; Index 2: Disulfide/total thiol×100; Index 3: Native thiol/total thiol×100.

brought superoxide dismutase, catalase, and glutathione levels to normal levels. Administration of lesinurad and allopurinol resulted in a significant reduction in serum levels of uric acid, blood urea nitrogen, xanthine oxidase activity, catalase, glutathione peroxidase, and inflammatory cytokines (IL-1β and TNF-a) reported in hyperuricemic mice. Both partially reversed oxonate-induced changes in renal mURAT-1, mGLUT-9, mOAT-1, and mOAT-3 expressions and changes in the immunoreactivity of TGF-β1 resulted in increased renal uric acid secretion and excretion. Combined administration of Lesinurad and ALP restored all the altered parameters in a synergistic manner, improving kidney function in the hyperuricemic mouse model used [29]. In another study, a decrease in ROS and proinflammatory cytokines was detected in mice treated with hyperuricemic agents [30]. In another study, it was determined that Ginsenoside R1, an antioxidant agent, reduced inflammation and gouty arthritis induced by MSU [31]. All these previous studies support our results.

Synoviocytes are known to express antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase [32]. This situation may cause an increase in antioxidant defense to help reduction of disulfide groups. As the disease duration increases, an increase in native thiols may have occurred as a defense mechanism against the increase in oxidation. The increase in both disulfide and native thiol may have indirectly caused an increase in total thiols.

To our knowledge, this is the first report in the literature to evaluate dynamic thiol-disulfide homeostasis and IMA together with common markers of inflammation in gout patients. The correlation of these oxidative stress markers with inflammation markers clearly demonstrates the importance of research. In addition, dynamic thiol-disulfide homeostasis was evaluated for the first time in gout and together with IMA, which was previously analyzed in various diseases. This study shows the role of oxidative damage in gout patients. Antioxidant support to active gout patients can be a supportable treatment approach. The results of this study revealed that oxidative stress is closely related to the severity of gout and inflammation.

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Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by The Ankara City Hospital Number 2 Clinical Research Ethics Committee (No: E2-21-402, Date: 18/05/2021).

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