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The role of thiol-disulfide homeostasis in gouty arthropathy

Tiyol-disülfit homeostazının gut artropatisindeki rolü

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Abstract

Objective: Gout, the most common form of crystal-induced arthritis, is characterized by the accumulation of monosodium urate crystals within the joints. This study aimed to examine thiol-disulfide homeostasis in patients with gouty arthropathy during periods of acute attack and remission.

Methods: A novel spectrophotometric technique was employed to assess native thiol (NT) and disulfide levels in gout patients and age- and sex-matched healthy controls. A total of 90 patients and 86 healthy individuals were evaluated using clinical and laboratory data extracted from their medical records.

Results: The findings demonstrated that NT and total thiol (TT) levels in patients were significantly lower than in controls (p<0.001 for both). No significant differences in NT and TT levels were observed between acute attacks and remission periods (p>0.05).

Conclusion: Alterations in thiol-disulfide homeostasis were evident in gout patients; however, these changes did not vary between periods of acute attack and remission.

Keywords: Gout, thiol, disulfide, oxidative stress, thiol-disulfide homeostasis

Öz

Amaç: Gut, kristal artropatinin en yaygın şeklidir ve eklemlerde monosodyum ürat kristallerinin birikmesinden kaynaklanır. Bu çalışmanın amacı, gut artropatisi olan hastalarda atak veya ataksız dönemde tiyol-disülfit dengesini araştırmaktır.

Yöntem: Gut hastalarında ve yaş-cinsiyet eşleştirilmiş sağlıklı kontrol grubunda doğal tiyol (DT) ve disülfit seviyelerini ölçmek için yeni geliştirilen bir spektrofotometrik yöntem kullanıldı. Toplam 90 gut hastası ve 86 sağlıklı kontrol incelendi. Klinik ve laboratuvar verileri tıbbi kayıtlardan elde edildi.

Bulgular: Gut hastalarında DT (p<0,001) ve toplam tiyol (TT) (p<0,001) seviyeleri sağlıklı kontrollere kıyasla anlamlı derecede düşüktü. Gut hastalarında atak ve ataksız dönemlerle DT ve TT düzeyleri arasında anlamlı bir fark yoktu (p>0,05).

Sonuç: Tiyol-disülfit homeostazisi gut hastalarında değişmekte, ancak atak döneminden etkilenmemektedir.

Anahtar Kelimeler: Gut, tiyol, disülfit, oksidan stres, tiyol-disülfit homeostazı

Introduction

Gout is a persistent inflammatory condition characterized by heightened uric acid levels and the saturation of monosodium urate (MSU) crystals in the patient's joints and adjacent tissues. This condition represents the most prevalent form of inflammatory arthritis among adults, particularly males, and its prevalence is surging worldwide, ranging from 1% to 3%.[1] In instances where the serum urate concentration surpasses 6.8 mg/dL, the precipitation of urate within joints and other tissues becomes a possibility. The accumulation of MSU in the intra-articular space activates inflammatory cytokines, which, in turn, results in the accumulation of macrophages and neutrophils. This series of events ultimately leads to the development of gouty arthritis.[2,3]

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Beyond the inflammatory process, the earliest event linked with gout may be oxidative stress (OS), which involves an imbalance between reactive oxygen species (ROS) and antioxidant mechanisms.[4] The oxidative state is induced by the generation of ROS and pro-inflammatory cytokines.[5] Previous *in vivo* research showed that elevated uric acid levels have the capacity to trigger endothelial dysfunction, manifesting in anti-proliferative effects on endothelial cells and impaired nitric oxide bioavailability.[6,7] The pivotal enzyme in this process, xanthine oxidase (XO), is key to ROS production, and the inhibition of XO with allopurinol has been demonstrated to enhance cardiovascular function.[8,9]

Dynamic thiol-disulfide homeostasis is key to antioxidant protection, detoxification, signal transduction, apoptosis, enzymatic activity, regulation of transcription factors, and cellular signaling mechanisms.[10,11] Thiols are functional groups found in the structure of major proteins, with the highest thiol levels in blood plasma found in albumin and other proteins. Thiols react with reactive oxygen radicals to oxidize and scavenge these radicals, thereby preventing tissue damage. Subsequent to this oxidation, disulfide bonds are formed.[12,13] It should be noted that these structures can be converted back to thiols. The measurement of thiol-disulfide levels offers an indirect indication of OS levels. Erel and Neselioglu[14] have recently introduced a novel spectrophotometric approach for measuring thiol-disulfide, characterized by its simplicity and cost-effectiveness. The method involves the quantification of native thiol (NT), total thiol (TT), and disulfide levels and the calculation of the relative proportions of these molecules [e.g., disulfide/native thiol (DNT), disulfide/total thiol (DTT), and native thiol/total thiol (NTT)].

The present study attempts to examine the thiol-disulfide equilibrium in patients with gout arthropathy during periods of acute attack and remission.

Materials and Methods

Sample

For this cross-sectional study, we considered data from 90 patients (77 males, 13 females) and 86 healthy subjects (73 males, 13 females). The patient group consisted of individuals diagnosed with gouty arthritis according to the 2015 ACR/EULAR gout classification criteria in our rheumatology clinic.[15] Patients who had not experienced a gout attack for at least six months were considered to be in remission. The control group comprised age- and sex-matched healthy individuals with no known chronic conditions.

Patients with the following conditions were excluded from the study: acute kidney problems, cardiovascular events, stroke, uncontrolled hypertension, malignancy, any infectious disease, and urgent medical conditions (e.g., respiratory failure due to interstitial disease) on the day of their evaluation at the outpatient clinic.

Blood Sampling

Blood samples were obtained from patients and controls after a 12-hour fast during the attack-free period. A similar protocol was applied to patients within the first 24 hours of an acute attack. We utilized 10 mL plain tubes containing ethylenediaminetetraacetic acid (EDTA) and 2 mL vacuum tubes for sampling. The samples were centrifuged at 1,500 g for 10 minutes.

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were measured within 3 hours of collection. Serum samples intended for thiol and disulfide analyses were meticulously stored at -80 °C until analysis. The same serum samples were used in the same session to measure NT, TT, and disulfide levels.

Biochemical Analysis

Serum NT, TT, and disulfide levels (µmol/L) were measured using a novel, affordable spectrophotometric technique.[14] Briefly, NT levels were initially assessed after the serum reacted with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) without any procedural modifications. Dynamic disulfide bonds in the samples were then reduced using sodium borohydride ($NaBH_4$) to measure TT levels, releasing free functional thiol groups. Formaldehyde was used to eliminate any unused $NaBH_4$, and reduced and native TT groups were measured following the reaction with DTNB.

The difference between NT and TT levels was calculated, and the amount of disulfide bonds was determined by subtracting NT from TT and dividing the result by two. Additionally, we calculated the ratios of DNT, DTT, and NTT.

For CRP measurements, we used the immunoturbidimetric method with the Beckman Coulter AU5800 clinical chemistry system (reference CRP <5 mg/L, Beckman Coulter, Inc., Brea, CA, USA). ESR levels were measured using the Alifax ESR analyzer system with the modified Westergren method (reference ESR <20 mm/h, Alifax, Polverara, Italy).

Statistical Analysis

We analyzed the data using SPSS version 18.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was employed to assess the normality of data distribution. Data are presented as mean (M), standard deviation, number (n), percentage (%), and range (minimum-maximum).

An independent samples t-test was used for parametric data, while the Mann-Whitney U test was applied for non-parametric data. Categorical variables were compared using chi-square analysis. Pearson's and Spearman's correlation analyses were used to assess relationships between continuous variables. A p-value <0.05 was considered statistically significant.

Ethics Statement

The protocol for this prospective case-control study was approved by the Institutional Review Board for Human Research of Ankara Numune Training and Research Hospital. All participants provided written informed consent before participating in the study.

Results

Age (55.2±13.8 years *vs.* 53.1±13.1 years, p>0.05) and gender distribution (M/F, n=77/13 *vs.* 72/14, p>0.05) were statistically similar between the groups. Table 1 presents the clinical and demographic characteristics of the patient group.

Table 1. Patients' demographic and clinical characteristics

Characteristics	Values
Gender (male/female)	77/13
Age, years	55.2±13.8 (24-82)
Disease duration, months	53.3±59.8 (0-480)
Number of attacks, annual	3.01±1.99 (0-8)
Patients with attack (n, %)	37 (41.1)
Subcutaneous tophi (n, %)	6 (6.7)
Medication	
Allopurinol	50 (55.6)
Febuxostat	4 (4.4)
Colchicine	16 (17.7)
Steroid	10 (11.1)
Colchicine + steroid	10 (11.1)
Urate lowering therapy (n, %)	54 (60%)

The findings revealed that NT, TT, and NTT levels were significantly lower in the patient group compared to control subjects. DNT, DTT, CRP, and ESR levels were significantly higher in patients. Although disulfide levels were elevated in patients, this increase did not reach statistical significance (p>0.05; Table 2).

Table 3 presents a comparative analysis of TT, NT, CRP, ESR, and disulfide levels in patients based on attack status. No significant differences were found in NT, TT, or disulfide levels between attack and remission periods (p>0.05). However, CRP and ESR levels were significantly higher in patients experiencing acute attacks compared to those in remission.

Correlation analyses showed that age was negatively correlated with NT levels (r=-0.341, p=0.000), TT levels (r=-0.336, p=0.000), and NTT (r=-0.188, p=0.013), but positively correlated with DNT (r=0.188, p=0.013) and DTT (r=0.188, p=0.013). No significant correlation was found between age and disulfide levels.

CRP levels were inversely correlated with NT (r=-0.252, p=0.017), TT (r=-0.233, p=0.027), and NTT (r=-0.213, p=0.044), while showing a positive correlation with DNT (r=0.213, p=0.044) and DTT (r=0.213, p=0.044). However, CRP levels did not significantly correlate with disulfide levels.

Additionally, disulfide parameters were not associated with disease duration, colchicine dose, allopurinol dose, ESR levels, or thiol levels.

Discussion

Gouty arthritis is an inflammatory condition arising from the deposition of MSU crystals within the joints. The secretion of various cytokines, prostanoids, chemotactic factors, and other proteins is induced by MSU crystals. This inflammatory mechanism is amplified through several pathways, including the recruitment of inflammatory cells, upregulation of adhesion molecules, and stimulation of the acute phase response.[16,17] Persistent, chronic inflammation

Table 2. Laboratory results of the patient and control group

Variables	Gout (n=90)	Control (n=86)	p-value
NT, µmol/L	239.9±65.3	457.2±54.6	0.00
TT, µmol/L	281.4±67	494.8±54.2	0.00
Disulfide, pmol/L	20.7±6.3	18.7±7.6	0.054
NTTx100	84.4±5.7	92.3±3	0.00
DNTx100	9.5±4.4	4.2±1.7	0.00
DTTx100	7.7±2.8	3.8±1.5	0.00
ESR, mm/h	22.7±14.5	7.52±4.29	0.00
CRP, mg/L	19.8±30.6	2.75±1.67	0.00

CRP: C-reactive protein, DNT: Disulfide/native thiol, DTT: Disulfide/total thiol, ESR: Erythrocyte sedimentation rate, NT: Native thiol, NTT: Native thiol/total thiol, TT: Total thiol

Table 3. Laboratory results of gout patients by attack status

Variables	Attack (n=53)	Attack-free (n=37)	p-value
NT, µmol/L	230.4±81.7	246.4±50.6	0.694
TT, µmol/L	270.5±84.2	289.1±51.3	0.715
Disulfide, pmol/L	20±6.2	21.3±6.4	0.463
NTTx100	83.6±6.9	84.9±4.7	0.468
DNTx100	10.2±5.5	9±3.4	0.468
DTTx100	8.1±3.4	7.5±2.3	0.468
ESR, mm/h	30.5±16.7	17.2±9.7	0.00
CRP, mg/L	38.6±40.4	6.7±6.5	0.00

CRP: C-reactive protein, DNT: Disulfide/native thiol, DTT: Disulfide/total thiol, ESR: Erythrocyte sedimentation rate, NT: Native thiol, NTT: Native thiol/total thiol, TT: Total thiol

eventually leads to OS and oxidative tissue damage.[18] A previous study highlighted the significance of OS and ROS in stimulating leucine-rich repeat and pyrin domain-containing protein inflammasomes induced by MSU crystals.[19]

Thiols are critical mediators in mitigating OS, with the capacity to safeguard against cellular damage by forming disulfide bridges that act as covalent bonds.[20] Consequently, thiol levels are considered significant markers of antioxidant capacity within the metabolic system. Thiol biochemistry has seen substantial growth in both fundamental and applied biological sciences, and since 1979, the assessment of sulfhydryl groups has commonly utilized DTNB as a standard protocol.[21] In this context, we aimed to evaluate serum thiol-disulfide homeostasis in gout patients using the spectrophotometric technique developed by Erel and Neselioglu.[14] Consistent with recent findings, our results showed that serum TT and NT levels were diminished in gout patients compared to healthy controls. Although disulfide levels correlated with CRP levels, they did not show an association with ESR or leukocyte counts.

A growing body of research has explored the interplay between chronic diseases (e.g., rheumatological conditions) and OS. However, there is limited literature on thiol-disulfide homeostasis in gout patients during periods of acute attack and remission. The study by Dogru et al.[22] made a notable contribution by demonstrating significantly reduced TT levels in ankylosing spondylitis (AS) patients. Similarly, Arpa et al.[23] observed significantly diminished TT and NT levels, alongside increased disulfide levels, in AS patients compared to controls. In both groups, ESR was negatively correlated with NT and TT levels, and high-sensitivity CRP (hs-CRP) levels showed similar negative correlations in patients with highly active AS. Serdaroğlu et al.[24] also reported significantly lower NT and TT levels in rheumatoid arthritis patients compared to healthy controls.

In another study, Omma et al.[25] found that dynamic thiol-disulfide homeostasis shifted towards disulfide formation due to thiol oxidation in patients with Familial Mediterranean fever. Additionally, juvenile idiopathic arthritis (JIA) patients demonstrated reduced plasma thiol levels, particularly during active disease periods. The researchers suggested that decreased thiol levels might play a critical role in the pathogenesis of JIA and that inflammatory diseases negatively impact antioxidant systems during heightened disease activity.[26]

In clinical practice, the severity of inflammation in various inflammatory conditions is typically assessed by measuring CRP and ESR levels. However, there is a lack of specific biomarkers for disease activity in gout. While serum uric acid is not a reliable predictor of flares or a diagnostic biomarker for gout, CRP remains a widely accepted marker of inflammation during gout flares. These findings underscore the role of OS in the inflammatory response in gout patients. Moreover, our results suggest that thiol-disulfide homeostasis could serve as a promising new biomarker for gout, though further studies are necessary to validate its cost-effectiveness in clinical settings.

Patients with gouty arthritis may benefit from adjuvant antioxidant-rich diets or treatments to enhance their antioxidant status. However, these findings require confirmation in larger cohorts of gout patients.

Study Limitations

This study has certain limitations. Its cross-sectional design and relatively small sample size (n=90) restrict the generalizability of the results. Additionally, OS levels can be influenced by several factors, such as lifestyle and dietary habits, which were not addressed in this study. Despite these limitations, this research offers valuable insights by examining thiol as a distinct biomarker of OS in gout patients.

Conclusion

This study demonstrates that thiol-disulfide homeostasis is disrupted in gout patients, with no significant changes observed between acute attack and remission periods.

Ethics

Ethics Committee Approval: The protocol for this prospective case-control study was approved by the Institutional Review Board for Human Research of Ankara Numune Training and Research Hospital.

Informed Consent: All participants provided written informed consent before participating in the study.

Footnotes

Authorship Contributions

Concept: S.C.S., A.O., Ö.E., Design: S.C.S., A.O., S.N., Data Collection and Processing: S.C.S., S.Y.Ç., S.N., Ö.E., Analysis or Interpretation: S.C.S., A.O., S.N., Ö.E., Literature Search: S.C.S., S.Y.Ç., A.O., Writing: S.C.S., S.Y.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the national health and nutrition examination survey 2007–2008. Arthritis Rheum. 2011;63:3136-41.
2. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440:237-41.
3. Martillo MA, Nazzal L, Crittenden DB. The crystallization of monosodium urate. Curr Rheumatol Rep. 2014;16:400.
4. Afonso V, Champy R, Mitrovic D, Collin P, Lomri A. Reactive oxygen species and superoxide dismutases: role in joint diseases. Joint Bone Spine. 2007;74:324-9.
5. Peng YJ, Lee CH, Wang CC, Salter DM, Lee HS. Pycnogenol attenuates the inflammatory and nitrosative stress on joint inflammation induced by urate crystals. Free Radic Biol Med. 2012;52:765-74.
6. Corry DB, Eslami P, Yamamoto K, Nyby MD, Makino H, Tuck ML. Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. J Hypertens. 2008;26:269-75.
7. Kanellis J, Kang DH. Uric acid as a mediator of endothelial dysfunction, inflammation, and vascular disease. Semin Nephrol. 2005;25:39-42.
8. Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. Lancet. 2010;375:2161-7.
9. Rekhraj S, Gandy SJ, Szwejkowski BR, et al. High-dose allopurinol reduces left ventricular mass in patients with ischemic heart disease. J Am Coll Cardiol. 2013:61:926-32.
10. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emergingroles in cellsignaling. Biochem Pharmacol. 2006;71:551-64.
11. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, andapoptosis. Free Radic Biol Med. 2010:48:749-62.
12. Yuksel M, Ates I, Kaplan M, et al. The dynamic thiol/disulphide homeostasis in inflammatory bowel disease and its relation with disease activity and pathogenesis. Int J Colorectal Dis. 2016;31:1229-31.
13. Kundi H, Ates I, Kiziltunc E, et al. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. Am J Emerg Med. 2015;33:1567-71.
14. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014;47:326-32.
15. Neogi T, Jansen T, Dalbeth N, et al. 2015 gout classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2015;74:1789-98.
16. Dalbeth N, Haskard DO. Mechanisms of inflammation in gout. Rheumatology (Oxford). 2005;44:1090-6.
17. Scanu A, Oliviero F, Ramonda R, Frallonardo P, Dayer JM, Punzi L. Cytokine levels in human synovial fluid during the different stages of acute gout: role of transforming growth factor β1 in the resolution phase. Ann Rheum Dis. 2012;71:621-4.
18. Lagnoux D, Darbre T, Schmitz ML, Reymond JL. Inhibition of mitosis by glycopeptide dendrimer conjugates of colchicine. Chemistry. 2005;11:3941-50.
19. Punzi L, Scanu A, Ramonda R, Oliviero F. Gout as autoinflammatory disease: new mechanisms for more appropriated treatment targets. Autoimmun Rev. 2012;12:66-71.
20. Itani HA, Dikalova AE, McMaster WG, et al. Mitochondrial cyclophilin D in vascular oxidative stress and hypertension. Hypertension. 2016;67:1218-27.
21. Ellman G, Lysko H. A precise method for the determination of whole blood and plasma sulfhydryl groups. Anal Biochem. 1979;93:98-102.
22. Dogru A, Balkarli A, Cetin GY, et al. Thiol/disulfide homeostasis in patients with ankylosing spondylitis. Bosn J Basic Med Sci. 2016;16:187-92.
23. Arpa M, Şen B, Beyazal MS, Erel O. Evaluation of thiol-disulfide homeostasis in active ankylosing spondylitis patients. Reumatol Clin (Engl Ed). 2021;17:S1699-258X(21)00118-2.
24. Serdaroglu Beyazal M, Arpa M, Devrimsel G, Yıldırım M, Erel O, Erdogan T. Thiol/disulphide homeostasis in patients with rheumatoid arthritis: a potential link between disease activity and preclinical atherosclerosis. Acta Reumatol Port. 2021;46:23-31.
25. Omma A, Sandikci SC, Kücüksahin O, Alisik M, Erel O. Can the thiol/disulfide imbalance be a predictor of colchicine resistance in familial mediterranean fever? J Korean Med Sci. 2017;32:1588-94.
26. Altinel Acoglu E, Erel O, Yazilitas F, et al. Changes in thiol/disulfide homeostasis in juvenile idiopathic arthritis. Pediatr Int. 2018;60:593-6.