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Oxidative stress in patients with osteoarthritis with infectious urinary tract: Approach through gene network analysis, urinary myeloperoxidase activity, and the oxidant/antioxidant status

İlter Demirhan[1]*, Erkan Öner[2] & Ergül Belge Kurutas[3]

[1]Biomedical Device Technology Program, Department of Electronic Automation, Vocational School of Health Services, Harran Universit,y Sanlıurfa-63300, Turkiye
[2]Department of Biochemistry, Faculty of Pharmacy, Adıyaman University, Adıyaman-02030, Turkiye
[3]Department of Medical Biochemistry, Faculty of Medicine, Sutcu Imam University, Kahramanmaras-46050, Turkiye

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Determining MPO activity and oxidant/antioxidant (MDA, SOD and CAT) levels in the urine of osteoarthritis patients with urinary infection was also performed for the first time to reveal the targets of gene enrichment network analysis of osteoarthritic and urinary system-related genes. In addition, our other main goal was to learn that the use of computer-aided designs in this study and other studies could help the target. Genes from patients with osteoarthritis and urinary infections were retrieved from DisGENET. A venn Diagram was drawn for genes from DisGENET, and gene network analysis for common genes was done with SRplot. 31 patients with concomitant osteoarthritis and urinary tract infection, 17 patients with osteoarthritis, 15 patients with urinary tract infection and 25 healthy controls were included in the study. Urinary MPO, catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were examined in a spectrophotometer as indicators of oxidant and antioxidant status. Gene network analysis was performed for the first time for urinary infection and osteoarthritis, and 50 common genes were found. These common genes appear to be associated with arthritis, according to the KEGG pathway result. In the osteoarthritis group, MPO activity and MDA levels were higher, and CAT and SOD levels were lower (P <0.001). CAT, SOD, MPO activity and MDA levels were higher in the urinary tract infection group compared to the control group (P <0.05). MPO activity, and MDA levels were found to be higher in patients with concomitant osteoarthritis and in the group with urinary tract infection, and CAT and SOD activities were found to be significantly lower in the group with only tract infection (P <0.001). This study revealed that the group with osteoarthritis and urinary tract infection showed different inflammatory reactions and caused an imbalance in the oxidant/antioxidant status. In addition, it was evaluated for the first time by network analysis and it was determined that which genes it was associated with and that these genes would lead other studies within the scope of this target.

Keywords: Gene set enrichment analysis (GSEA), KEGG Pathway

Free radicals are atoms or molecules with unpaired electrons that can be formed during many physiological and pathological reactions. These unpaired molecules are highly reactive and initiate reactions that will destroy important structures such as proteins, lipids and nucleic acids in the cell. Therefore, research in the physiopathology of many diseases is becoming more and more important day by day. It is widely accepted that these radicals are also responsible for osteoarthritis diseases[1].

Osteoarthritis (OA) is the most common chronic rheumatic diseases and is the leading cause of pain and disability worldwide. It occurs as a result of the natural immune response as a result of micro and macrotrauma in mobile joints and progresses with low-intensity inflammation, cell stress and extracellular matrix destruction. If we consider the joint as an organ, osteoarthritis can occur in the pathology of all the structures that make up the joint. Although the inflammation is of low severity, many substances are released during this inflammation. Although it is not known which inflammation markers are effective in the diagnosis of osteoarthritis, there are many studies related to this[2]. There is increasing evidence for the presence of inflammation biomarkers in OA. Superoxide dismutase (SOD) and catalase (CAT) are probably the most widely used biochemical markers of systemic inflammation[2]. Earlier studies on myeloperoxidase (MPO) in synovial fluid, serum and urine have shown increased levels in

*Correspondence:
Phone: +90 5055582871
E-Mail: ilterdemirhan@harran.edu.tr
ORCID: 0000-0003-0054-7893

OA patients. Further, MPO is recommended as a biomarker for the early diagnosis of OA[3,4].

Urinary tract infections (UTIs) are a disease that threatens all age groups and is observed in young and old people. UTIs cause diseases such as sepsis and kidney failure, as well as having fatal consequences[5]. Early diagnosis and adequate treatment of UTI diseases are of great importance in terms of patient life[6].

Modern analysis of biological data is based on the consolidation of interactions between more and more genes, proteins, and other biological molecules into networks, in other words, interactoms. Often, the task is to characterize a new experimental or pathological condition through a set of genes with altered molecular properties[7].

Gene set enrichment analysis (GSEA) is a powerful tool for the interpretation of high-throughput expression studies such as mass spectrometry-based proteomics or next generation sequencing to identify insights into the biological processes or pathways underlying a particular phenotype. By acquiring an expression profile, the list of differentially expressed genes is sorted as a metric associated with the observed expression change. The ordered gene list is compared to a gene set, i.e., a list of genes known to be associated with a particular biological process, gene ontology (GO), molecular function, or pathway. The metric is required to calculate the enrichment score (ES), which indicates the degree of overrepresentation of a gene set at the extremes of the ranked list[8,9].

In this study, we explored the potential relationship between gene network analysis and oxidative stress damage processes in patients with osteoarthritis. Additionally, levels of MDA and MPO were measured to assess the oxidative stress damage in osteoarthritis patients with urinary infections. In addition, we measured the levels of antioxidant enzymes CAT and SOD as well.

Materials and Methods

The study included 31 patients (16 females, 15 males; between the ages of 55 and 70) with knee osteoarthritis and urinary infection (UTI+OA), 17 patients with osteoarthritis (OA) (7 females, 10 males; between the ages of 51 and 70), and 15 subjects with only urinary infection (UTI) (6 females, 9 males; between the ages of 53 and 67), and 25 healthy (12 females, 13 males; between the ages of 50 and 69), including the control group. Ethics committee approval was obtained from the ethics committee unit on July 12, 2020, in accordance with the Declaration of Helsinki. A voluntary consent form was signed by the individuals involved in the study.

Midstream spot urine samples taken under sterile conditions were inoculated on 5% sheep blood agar and Mac Conkey agar media, and after 24 h of incubation at 37°C, $\geq 10^5$ CFU/mL growth was detected in the samples. Urinary system infectious agents were detected by routine biochemical methods and the VITEK (BioMeriéoux, France) system.

As for indicators of oxidant/antioxidant status, catalase (CAT), superoxide dismutase (SOD) levels, malondialdehyde (MDA) levels, and also MPO as an enflammatuar marker in urine, were measured by the spectrophotometric method. Antioxidant and oxidant levels were measured at room temperature (25°C).

Biochemical analyses

Urine samples

Urine samples brought to the laboratory were diluted 1:50 with serum physiologic (0.9% NaCI) in order to perform biochemical analysis. Urine creatinine levels were measured and kept under constant control.

Measurement of MPO SOD and CAT activity

The basic principle of the method is based on the oxidation of odianisidine (3,3'- dimethoxybenzidine). MPO activity was calculated by measuring the absorbance of this oxidized product at 460 nm for 10 min. The unit of MPO activity is given as U/L[10]. The Fridovich method was used for SOD determination[10]. SOD activity was expressed as U/L. CAT activity was determined in the urine sample by the Beutler method[11]. CAT catalyzes the breakdown of hydrogen peroxide (H_2O_2). The rate of degradation of H_2O_2 by CAT is measured spectrophotometrically using light absorption at 230 nm. CAT activity was expressed as U/L.

Measurement of malondialdehyde

For determination of MDA, we used Ohkawa method[12]. The MDA concentration was expressed as nmol/mL.

Statistical analysis

The data were analyzed using the SPSS 25.0 package program. P <0.05 was accepted as the statistical significance level. If a group is compared with the control group and there is a significant difference, it is indicated with an (*).

Gene network analysis

OA and UTI related targets were retrieved from the online DisGeNET database (https://www.disgenet.org).

Table 1 — SOD, CAT enzyme activities and MDA levels in Control, UTI Infection and Infection with UTI-OA groups

	CAT (U/L)	P	SOD (U/L)	P	MDA (nmol/mL)	p
UTI+OA Group	0.94±0.06*	0.01	5.53±0.46*	0.04	4.27±0.62**	0.002
UTI Group	0.36±0.086*	0.02	1.94±0.84*	0.02	3.22±0.42**	0.001
OA Group	0.25±0.22	0.01	1.87±0.37	0.03	3.75±0.67**	0.003
Control Group	0.17±0.05	0.01	1.47±0.48	0.01	1.99±0.44**	0.002

[Results are given as mean and standard deviation. Statistically significant (P <0.05) *higher; and **lower than the control]

Table 2 — UTI and OA common genes list

POMC, IL1B, IL1A, IL18, TLR4, IL6, CRP, CXCL8, TNF, IL10, ALB, TGFB1, ACE, VEGFA, NLRP3, TCF21, CCR2, CCL2, NHS, VDR, CORO7, TLR2, PTGS2, PLAU, CD44, CD36, TLR9, TRPV1, BCL2, REN, TSPO, CCL20, HSPA14, ESR1, EFEMP1, MTCO2P12, CHRM3, CHI3L1, MMP9, MMP1, IL22, SERPINB2, MYD88, COX2, AR, HIF1A, HSPD1, CXCL10, IL17A, IL1RN

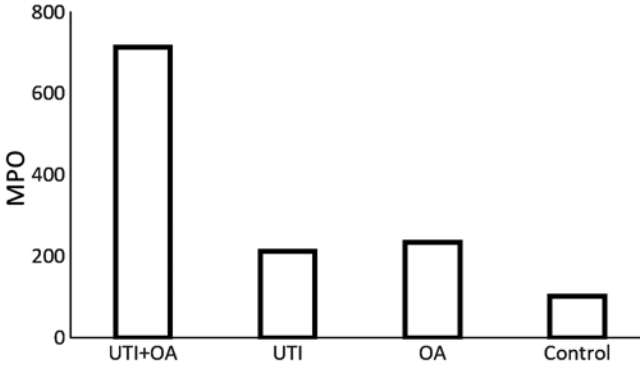

Fig. 1 — MPO level in the control group, urinary tract infection, urinary tract infection-osteoartrit

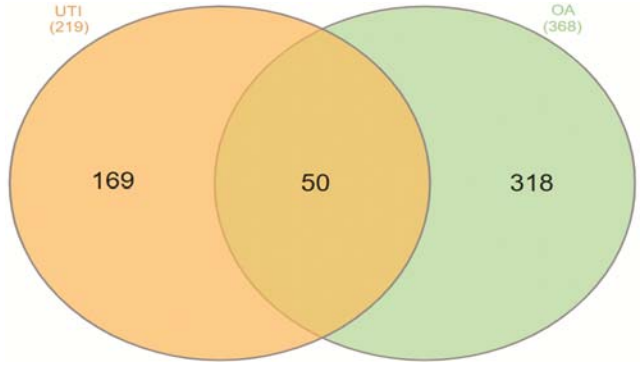

Fig. 2 — Venn diagram of UTI and OA genes

We used the InteractiVenn online tool to identify common targets of OA and UTI[13]. A network analysis of 50 common genes extracted from the Venn diagram was performed. Analyzed with "https://www.bioinformatics.com.cn/en", a free online platform for data analysis and visualization[14,15]

Results

MPO activity and MDA levels were found to be significantly higher, while CAT and SOD activities were found to be low in the osteoarthritis group (P <0.001). CAT, SOD, and MDA levels with MPO activities were found to be higher in the urinary infection group compared to the control group (P <0.05). In addition, the levels of MDA and MPO

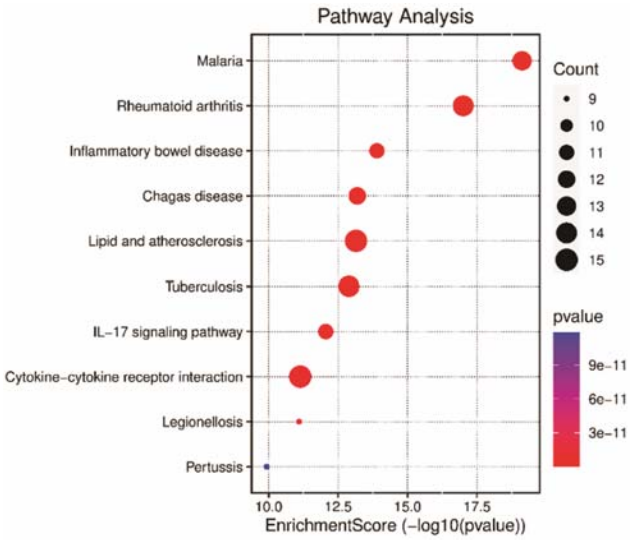

Fig. 3 — Pathway enrichment plot for OA and UTI common genes

activities in the osteoarthritis group with urinary infection were significantly higher compared to the control group, while the CAT and SOD levels were significantly lower in the osteoarthritis group with urinary infection compared to the group with only urinary infection (P <0.001) (Table 1) (Fig. 1).

UTI and OA genes were obtained from DiSGENET and Venn diagram was drawn (Fig. 2). According to the Venn diagram, UTI and OA have 50 genes in common. According to the Venn Diagram results, the OA and UTI genes taken from Dissgenet are shown in Table 2 as the list of 50 common genes. The dots represent term enrichment by colour coding: red indicates high enrichment, blue indicates low enrichment. The sizes of the dots represent the percentage of each row (GO category). These results indicate diseases in which there is high expression of genes associated with the common 50 genes of OA and UTI. It shows that it provides a good prognosis for rheumatoid arthritis for our study. This analysis provides valuable information for further research (Figs. 3 and 4). The KEGG pathway map of rheumatoid arthritis is given in Fig. 5.

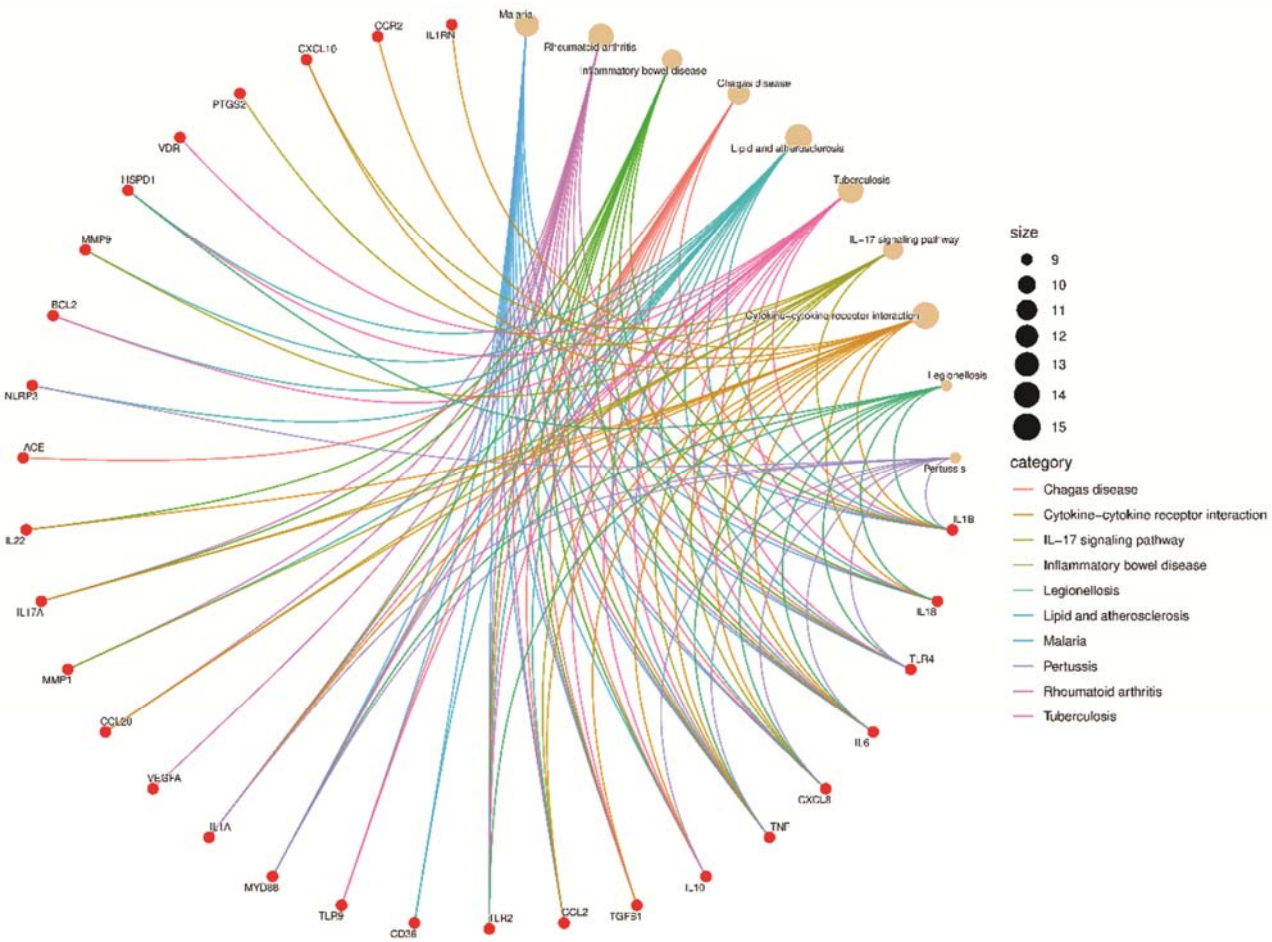

Fig. 4 — Relationship of pathways related to OA and UTI common genes with genes

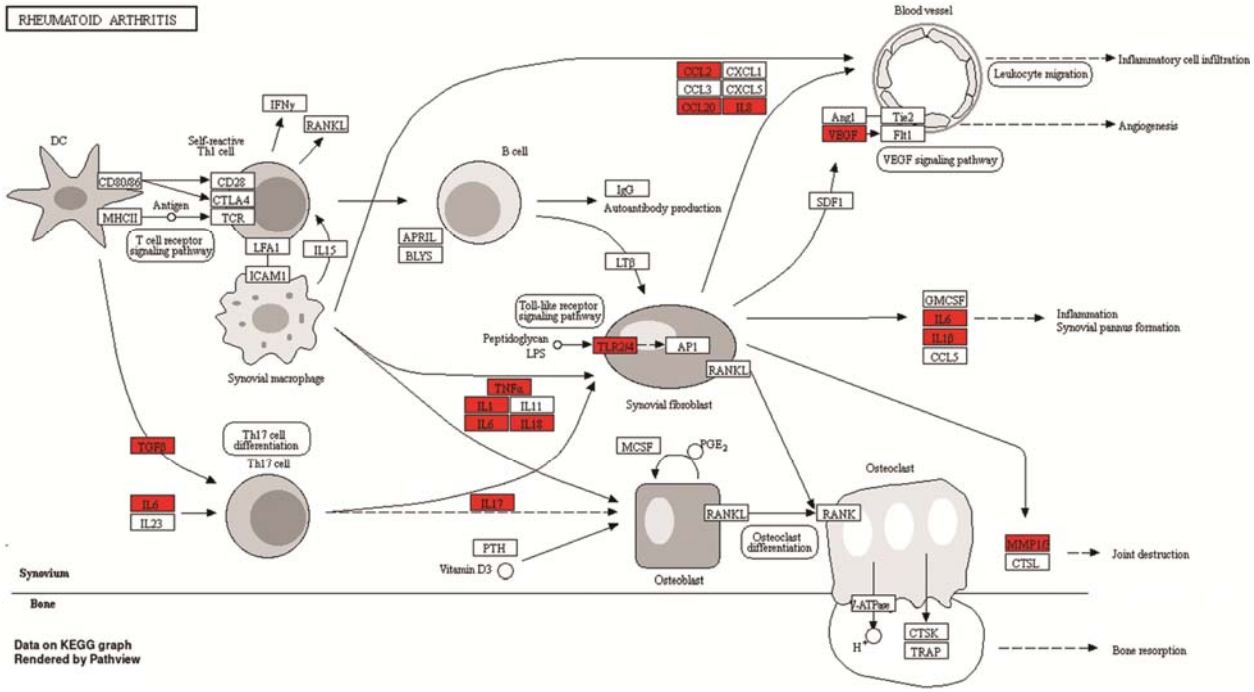

Fig. 5 — KEGG pathway of rheumatoid arthritis

Discussion

When we compared the control group in our study, while the MPO level increased very much in UTI+OA, it was observed that it increased only slightly in the OA and only UTI groups. High MPO activities are suggestive of pathogenicity in UTI+OA, OA-only, and UTI-only groups. In our evaluation in terms of oxidative stress, while the MDA level increased very much in the UTI+OA group compared to the control group, there was a lesser increase in only the OA and UTI groups. When we compared the control group, SOD levels were approximately found only in the UTI and only OA groups, while a significant increase was observed only in the UTI+OA group. When we make a comparison with the control group, there was a slight increase in CAT levels only in the OA and UTI groups, while a significant increase was observed in the UTI+OA group.

OA is the most common form of joint disease. It is the leading cause of chronic disability, especially in middle-aged and elderly populations[16,17]. However, the pathogenesis and progression of OA is still unclear. Bioinformatics based on gene relationships has been carried out in order to address this uncertainty. Gen enrichment analysis showed that inflammatory bone disease is associated with certain cytokines. These cytokines were associated with IL-1B, IL-8, IL-22 and TNF. These cytokines play an significant role in the mechanism of oxidative damage. IL-1 is a very important cytokine in the early stages of osteoarthritis, and chondrocytes have a high sensitivity to it, and can be considered as a go between the different structures of the joints[18].

Takahashi et al. determined that interlokin-8, an inflammatory chemokine in osteoarthritis, has an epigenetic regulated role[19]. Another study showed that IL-22 protects against osteoarthritis[20]. Several recent studies have shown that IL-1, IL-8, IL-22 and TNF cytokines play an important role in the mechanism of oxidative damage[21-23].

Pathophysiologically, OA is still not fully understood[24]. However, the journey to understanding the link between oxidative stress and OA is much more recent and goes back nearly 30 years. To date, it has been aimed at detecting biomarkers by conducting studies in both animal models and humans. A list of biomarkers routinely used in the diagnosis and treatment of osteoarthritis and the studies of these markers in patients[25,26].

Our study is the first to identify oxidative stress biomarkers in patients with urinary tract infections and urinary tract infections with osteoarthritis[27]. A limited number of studies show that there is an ongoing debate about which biomarkers should be used. It is suggested that oxidative stress biomarkers should be used for research purposes until more sensitive and specific tests are developed[28]. It is seen that GPx, SOD, CAT, GR, AE, NADPH ox, and MPO enzymes are evaluated in terms of enzymatic activity in patients with OA. It is seen that GPx is mostly studied in studies. Lower GPx levels were noted in patients with OA compared to controls in all studies[29]. Superoxide dismutase (SOD) is an endogenously formed metalloprotein enzyme that protects cells against the toxic effects of superoxide radicals. SOD catalyzes the conversion of superoxide to hydrogen peroxide. SOD activity measurement is a mechanism that makes research valuable because it acts as a primary protector in cells. In the study, CAT and SOD enzyme activities were found at lower levels in the osteoarthritis group with urinary infection compared to the control group. The low levels of these two enzymes suggest a correlation between ROS, OA, and urinary system diseases. In some studies, it is stated that SOD and CAT antioxidant enzyme levels are low in patients with OA and UTI[30]. Studies in which ROS production and oxidative stress are evaluated as high in patients with OA are reported in the literatüre[31]. There are many studies in the literature indicating that there is an increase in oxidative stress (decrease in SOD, CAT, and Gpx enzyme values) damage in the pathogenesis of OA.[32].

The reduction of important antioxidant systems such as SOD and CAT causes functional and mechanical failures and, thus degenerative destruction in cartilage tissue. There are similar studies emphasizing that the SOD enzyme, which we found at low levels in our study, similar to previous studies, can be used as a biomarker in patients with OA[33]. Because SOD activity is not affected by age, Paździor et al. showed low levels of SOD and CAT activity in older women with OA[32]. It is thought that CAT enzyme activity reduces the level of ROS in chondrocytes, reduces TNF-α-induced apoptosis, and contributes to the physiological remodeling of cartilage[34]. SOD enzyme activity was found to be higher in patients with OA compared to controls in only two of the six studies[35]. While no difference was observed between the control and OA patient groups

in most of the studies in terms of CAT activity[36,37], Ferreira et al[33]. reported decrased CAT activity levels in the plasma of patients with OA. Studies evaluating the antioxidant enzymes GR, AE, and NADPH ox in OA are limited. Studies in which NADPH ox and GR activity were evaluated lower in the patient group are available in the literatüre[38,39].

It has been stated that MPO activity may be a non-invasive indicator in patients with UTI. In the literature, there are many studies in which MPO activity increases due to UTI and OA[39]. On the contrary, Fonseca et al.[34] reported that MPO activity was decreased in patients with OA compared to controls. MDA is a frequently used biomarker for lipid peroxidation[35]. OA disease, which has effects on the breakdown and oxidation of collagen in cartilage, is characterized by high MDA values. In our study, MDA levels were found to be significantly higher in the osteoarthritis group (P <0.001). In addition, the levels of MDA in the osteoarthritis group with urinary infection were significantly higher compared to the control group. Garg *et al.*[40] showed that there was a 6.5% increase in MDA levels in patients with OA compared to the control group. Zhang *et al.*[41] reported that sodium hyaluronate treatment decreased MDA and TNF-α levels in patients with moderate and severe OA and provided a significant improvement in clinical symptoms. Weber *et al.*[42] also were studied with synovial cell cultures isolated from patients with OA, found that MDA levels were high. Low levels of MDA are observed in some segments of the geriatric population. According to the researchers, this situation is associated with the genetic presence of a protective component against oxidative stress[40]. In addition, thanks to the exogenous antioxidants to be applied externally, there is a decrease in the MDA level. In a study, lower MDA levels were shown in the group treated with hyaluronic acid in patients with OA compared to the control group[43]. It is also stated that turmeric and ginger extracts reduce MDA concentrations in patients with OA[43]. In addition to MDA, 8-isoprostane F2α, thiobarbituric acid reactive substances (TBARS), and malondialdehyde-acetaldehyde (MAA) have been investigated biomarkers of lipid peroxidation in OA patients. Blood TBARS levels were evaluated as higher in patients with OA compared to controls[32,44]. Studies have reported that 8-isoprostane F2α concentrations are higher in OA patients than in healthy controls[40,45].

Gene network analysis was performed for the first time, and as a result, 50 common genes associated with OA and UTI were found. According to the results of the network analysis of these genes, we think that UTI may be associated with the prognosis of OA due to the related genes between OA and UTI. In addition, when we look at the KEGG pathway, we see that there are genes associated with the oxidative damage mechanism.

Conclusion

In summary, oxidant and antioxidant levels were evaluated in patients with osteoarthritis who had urinary infection. Research results suggest that antioxidant enzymes can be used as biomarkers in patients with OA with urinary infection. Our study showed that there was a relationship between osteoarthritis and the urinary tract infection group. We showed a gene network relationship between the cytokines IL-1, IL-8, IL-22 and TNF. Since these cytokines play an important role in oxidative damage, it was thought that they would have an active role in patients with osteoarthritis patients with urinary tract infections. This study is the first to identify genes involved in oxidative damage and will lead to other studies.

Statement of ethics

This study was performed with the principles of the Declaration of Helsinki. This retrospective study was conducted at the Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey.

Conflict of Interest

Authors declare no competing interests.

References

1 Bolduc JA, Collins JA & Loeser RF, Reactive oxygen species, aging and articular cartilage homeostasis. *Free Radic Biol Med*, 132 (2019) 73.

2 Zhang W, Li R, Wang S, Mu F & Jia P, Effect of Chinese traditional herb *Epimedium grandiflorum C. Morren* and its extract Icariin on osteoarthritis via suppressing NF-kappaB pathway. *Indian J Exp Biol*, 51 (2013) 313.

3 Kurutas EB, Gumusalan Y, Cetınkaya A & Dogan E, Evaluation of method performance for oxidative stress biomarkers in urine and biological variations in urine of patients with type 2 diabetes mellitus and diabetic nephropathy. *Biol Proced Online*, 17 (2015) 1.

4 Paździor M, Kiełczykowska M, Kurzepa J, Luchowska-Kocot D, Kocot J & Musik I, The Oxidative Stress in Knee Osteoarthritis Patients. An Attempt of Evaluation of Possible Compensatory Effects Occurring in the Disease Development. *Medicina*, 55 (2019) 150.

5 Zahan OM, Serban O, Gherman C & Fodor D, The evaluation of oxidative stress in osteoarthritis. *Med Pharm Rep*, 93 (2020) 12.

6 Mancuso G, Midiri A, Gerace E, Marra M, Zummo S & Biondo C, Urinary Tract Infections: The Current Scenario and Future Prospects. *Pathogens*, 12 (2023) 623.

7 Jeggari A, Alekseenko Z, Petrov I, Dias JM, Ericson J & Alexeyenko A, EviNet: a web platform for network enrichment analysis with flexible definition of gene sets. *Nucleic Acids Res*, 2 (2018) 46.

8 Thomas PD, The gene ontology and the meaning of biological function. *Methods Mol Biol*, 1446 (2017) 15.

9 Turut H, Kurutas EB, Bulbuloglu E, Yasım A, Ozkaya M, Onder A & Imrek SS, Zinc aspartate alle-viates lung injury induced by intestinal ischemia-reperfusion in rats. *J Surg Res*, 151 (2009) 62-67.

10 Fridovich I, Superoxide dismutase. In: Advances in Enzymology and Related Areas of Molecular Biology, vol. 41 (Ed. Meister A; Wiley Online Library), 1974, 35. https://doi.org/10.1002/9780470122860.ch2.

11 Beutler E. *Red Cell Metabolism. A Manual of Biochemical Methods*. 3rd edn. (Grune & Stratton, Orlando, FL, USA), 1984.

12 Ohkawa H, Ohishi N & Tagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95 (1979) 351.

13 Hines M, Gomez-Contreras PC, Rodman S, Liman S & Coleman M, Lipid peroxidation regulates chondrocyte mitochondrial dynamics and cartilage injury response. *Free Radic Biol Med*, 180 (2022) 22.

14 Heberle H, Meirelles GV, da Silva FR, Telles GP, Minghim R, InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*, 16 (2015) 169.

15 Van den Belt M, Gilchrist C, Booth TJ, Chooi YH, Medema MH & Alanjary M, CAGECAT: The CompArative GEne Cluster Analysis Toolbox for rapid search and visualisation of homologous gene clusters. *BMC Bioinformatics*, 24 (2023) 181.

16 Zito A, Lualdi M, Granata P, Cocciadiferro D, Novelli A, Alberio T, Casalone R & Fasano M, Gene Set Enrichment Analysis of Interaction Networks Weighted by Node Centrality. *Front Genet*, 24 (2021) 577.

17 Zhang M & Wang J, Epigenet Osteoarthritic. *Genes Dis*, 2 (2015) 69.

18 Van den Bosch MHJ, Blom AB & van der Kraan PM, Inflammation in osteoarthritis: Our view on its presence and involvement in disease development over the years. *Osteoarthritis Cartilage*, 23 (2023) 4584. doi: 10.1016/j.joca.2023.12.005.

19 Takahashi A, de Andrés MC, Hashimoto K, Itoi E & Oreffo RO, Epigenetic regulation of interleukin-8, an inflammatory chemokine, in osteoarthritis. *Osteoarthritis Cartilage*, 23 (2015)1946.

20 Yi C, Yi Y, Wei J, Jin Q, Li J & Sacitharan PK, Targeting IL-22 and IL-22R protects against experimental osteoarthritis. *Cell Mol Immunol*, 18 (2021) 1329.

21 Yao Q, Wu X, Tao C, Gong W, Chen M, Qu M, Zhong Y, He T, Chen S & Xiao G, Osteoarthritis: pathogenic signaling pathways and therapeutic targets. *Signal Transduct Target Ther*, 8 (2023) 56.

22 Xie Z, Hou H, Luo D, An R, Zhao Y & Qiu C, ROS-dependent lipid peroxidation and reliant antioxidant ferroptosis-suppressor-protein 1 in rheumatoid arthritis: a covert clue for potential therapy. *Inflammation*, 44 (2021) 35.

23 Ansari MY, Ahmad N & Haqqi TM, Oxidative stress and inflammation in osteoarthritis pathogenesis: role of polyphenols. *Biomed Pharmacother*, 129 (2020) 110452.

24 Ambalayam S & Mathur R, Extremely low frequency magnetic exposure attenuates oxidative stress and apoptotic cell death in injured spinal cord of rats. *Indian J Exp Biol*, 60 (2022) 248.

25 Dong EY, Hu C, Huang C, Gao J, Niu W, Wang D, Wang Y & Niu C, Interleukin-22 Plays a Protective Role by Regulating the JAK2-STAT3 Pathway to Improve Inflammation, Oxidative Stress, and Neuronal Apoptosis following Cerebral Ischemia-Reperfusion Injury. *Mediators Inflamm*, 12 (2021) 662.

26 Agarwal A & Prabakaran SA, Mechanism, measurement and prevention of oxidative stress in male reproductive physiology, *Indian J Exp Biol*, 43 (2015) 963.

27 Liang Q, Huang Y & He S, Pathway centric analysis for single-cell RNA-seq and spatial transcriptomics data with GSDensity. *Nat Commun*, 14 (2023) 8416.

28 Zamudio-Cuevas Y, Martínez-Flores K, Martínez-Nava GA, Clavijo-Cornejo D & Fernández-Torres J, and Sánchez-Sánchez R. Rheumatoid Arthritis and Oxidative Stress: Rheumatoid arthritis and OA. *Cell Mol Biol*, 68 (2022) 174.

29 Li S, Yang X, Feng Z, Wang P, Zhu W & Cui S, Catalase enhances viability of human chondrocytes in culture by reducing reactive oxygen species and counteracting tumor necrosis factor-α- induced apoptosis. *Cell Physiol Biochem*, 49 (2018) 2427.

30 Wang X, Fan D, Cao X, Ye Q, Wang Q, Zhang M & Xiao C, The Role of Reactive Oxygen Species in the Rheumatoid Arthritis-Associated Synovial Microenvironment. *Antioxidants*, 11 (2022) 1153.

31 Koike M, Nojiri H, Kanazawa H, Yamaguchi H, Miyagawa K, Nagura N, Banno S, Iwase Y, Kurosawa H & Kaneko K, Superoxide dismutase activity is significantly lower in end-stage osteoarthritic cartilage than non-osteoarthritic cartilage. *PloS One*, 13 (2018) 9.

32 Pazdzior M, Kiełczykowska M, Kurzepa J, Luchowska-Kocot D, Kocot J & Musik I, The oxidative stress in knee osteoarthritis patients. An attempt of evaluation of possible compensatory effects occurring in the disease development. *Medicina*, 55 (2019) 150.

33 Li S, Yang X, Feng Z, Wang P, Zhu W & Cui S, Catalase enhances viability of human chondrocytes in culture by reducing reactive oxygen species and counteracting tumor necrosis factor-α- induced apoptosis. *Cell Physiol Biochem*, 49 (2018) 2427.

34 Ferreira HB, Melo T, Paiva A & Domingues MdR, Insights in the Role of Lipids, Oxidative Stress and Inflammation in Rheumatoid Arthritis Unveiled by New Trends in Lipidomic Investigations. *Antioxidants*, 10 (2021) 45.

35 da Fonseca LJS, Nunes-Souza V, Goulart MOF & Rabelo LA, Oxidative Stress in Rheumatoid Arthritis: What the Future Might Hold regarding Novel Biomarkers and Add-On Therapies. *Oxid Med Cell Longev*, 14 (2019) 7536805.

36 Bassu S, Zinellu A, Sotgia S, Mangoni AA, Floris A, Farina G, Passiu G, Carru C & Erre GL, Oxidative Stress Biomarkers and Peripheral Endothelial Dysfunction in Rheumatoid Arthritis: A Monocentric Cross-Sectional Case-Control Study. *Molecules*, 17 (2020) 3855.

37 Ciragil P, Kurutas EB & Miraloglu M, New markers: Urine xanthine oxidase and Myeloperoxidase in the early Detection of urinary tract infection. *Dis Markers*, 4 (2014) 2014.

38 Hunter DJ & Bierma-Zeinstra S, Osteoarthritis. *Lancet*, 393 (2019) 1745.
39 Qi Z, Zhu J, Cai W, Chunbiao L & Zongyu L, The role and intervention of mitochondrial metabolism in osteoarthritis. *Mol Cell* Biochem, 24 (2023) 101.
40 Garg MC, Chaudhary DP & Bansal DD, Effect of Vitamin E supplementation on diabetes induced oxidative stress in experimental diabetes in rats. *Ind J Exp Biol*, 43 (2005) 177.
41 Zhang X, Hou L, Guo Z, Genchun W, Jingting X, Zehang Z, Kai S & Fengjing G, Lipid peroxidation in osteoarthritis: focusing on 4-hydroxynonenal, malondialdehyde, and ferroptosis. *Cell Death Discov*, 9 (2023) 320.
42 Weber D, Stuetz W, Toussaint O, Chainiaux FD, Dolle MET, Jansen E, Gonos ES, Frenceschi C, Sikora E, Hervonen A, Breusing N, Sindlinger T, Villanueva NN, Burkle A & Grune T, Associations between specific redox biomarkers and age in a large European cohort. *Oxid Med Cell Longev*, 31 (2017) 117.
43 Cheng Q, Zhang T, Yaron S, Jiang HX, Sui, ZQ & Corke H, Phenolic profiles, antioxidant, and antiproliferative activities of turmenic (Curcuma Longa). *Ind Crop Prod*, 152 (2020) 112.
44 Anke JR & Cosimo DB, Osteoarthritis year in review 2023:Biology. *Osteoarthiritis Cartilage*, 2023 (2023) S1063.
45 Wen MT, Liang XZ & Luo D, Plasma lipids, alcohol intake frequency and risk of Osteoarthritis: a Mendelian randomization study. *BMC Public Health*, 23 (2023) 1327.