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Assessment of Thiol/Disulphide Homeostasis in Patients with Knee Osteoarthritis

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Our aim was to explore the thiol/disulphide homeostasis and the link with functional status in patients who have knee OA. Sixty knee OA patients and 50 healthy individuals were enrolled in this study. We measured serum levels of native thiol, total thiol and disulphide. In order to measure the alterations in functional status such tests as the Western Ontario, MacMaster Osteoarthitis index (WOMAC), walking test and Visual Analogue Scale (VAS) were utilized. The total thiol levels were higher in the control group than the knee OA patients ($P< 0.05$). Disulphide and disulphide/total thiol levels were significantly lower when control group were compared to knee OA patients ($P< 0.05$).Activity pain was negatively associated with native thiol levels ($P< 0.05$).Walking test scores were negatively correlated with the native thiol levels ($P< 0.05$) and positively correlated with disulphide levels ($P< 0.05$) in knee OA patients. In knee OA patients, no correlation was observed between thiol/disulphide parameters and WOMAC scores. Conclusion, thiol/disulphide homeostasis is impaired in patients with knee osteoarthritis. Disulphide level increased and thiol level decreased due to oxidative stress. Thiol/ disulphide homeostasis had not noticeable impact on the on functional status. Thiol/disulphide homeostasis may help to explain the pathogenesis of osteoarthritis.

Keywords: Functional status**,** Knee osteoarthitis, Oxidative stress

Osteoarthritis (OA) is a degenerative joint disease of unknown etiology that affects many different joints and, leads to disability in the population[1]. Most patients experience difficulties in their daily life activities and approximately one fourth have functional limitations, such as decreased joint motion, muscle atrophy and morning stiffness[2]. The pathogenesis of OA has not been completely understood yet, several genetic and environmental factors associated with the molecular pathway that contribute to the progression of the disease affect the pathogenesis of osteoarthritis[3]. Subchondral bone cartilage, synovial membrane, ligaments, and periarticular muscles are affected by OA. It is well known that articular cartilage changes play an important role in the development of OA[1]. .Irreversible deterioration of joint structure and cartilage loss leading to progressive deterioration are observed in OA pathogenesis[4]. Some patients with acute synovitis in OA have been found to have synovial proliferation and inflammatory changes[5]. In previous studies, reactive oxygen species (ROS) production has been suggested to increase in joint diseases such as OA and rheumatoid arthritis[6].

Thiols are a group compounds including sulfhydryl groups in their structures. The homeostasis of thiol/disulphide is one of the most important protection mechanisms opposite oxidative stress[7-9]. ROS is released into the area of inflammation along with proinflammatory factors in many joint diseases[10]. ROS has the capacity for oxidation and unfold proteins, playing role in the harmful effects of oxidative stress. Thiols due to oxidative stress may undergo an oxidation reaction to compose reversible disulphide bonds. Thus, dynamic thiol/disulphide balance status maintained[11]. Thiol disulphide homeostasis plays significant roles in antioxidant effect, apoptosis, signal transduction, detoxification, enzymaticactivities and cellular signaling mechanisms[9,12]. In our study, our aim was to compare the thiol disulphide homeostasis of knee OA patients and healthy individuals and to explore the link with functional status in knee OA patients.

Materials and Methods

Study population

Sixty knee OA patients and 50 healthy individuals were enrolled in this study. In line with American

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College of Rheumatology criteria for knee OA, our study included OA patients[13]. Patients have grade 2 or 3 degree knee osteoarthritis according to Kellgren and Lawrence criteria[14]. The knee OA patients received analgesic (paracetamol; 1500 mg/day) treatment.We got all participants' specified histories and fulfilled systemic and rheumatologic examinations. Patients and healthy individuals with a history of chronic diseases (diabetes mellitus, hypertension, obesity, hyperlipidemia, depression, autoimmune disease), alcohol and cigarette consumption, steroids and immunosuppressive drugs use for the last four weeks, the presence of available infections and regular vitamin medication were excluded. This study design was approved by The Recep Tayyip Erdogan University Research Ethics Committee and informed consent was provided by all participants.

Measurement procedures

By the method defined by Erel and Neselioglu[15], plasma total thiol, native thiol, and disulphide levels of all patients and volunteers were measured and then we assessed thiol/disulphide homeostasis. In this method initially, the thiol level in plasma was measured without pretreatment. This first result was regarded as native thiol. Next, a pretreatment process was performed using sodium borohydrate to reduce the dynamic disulfide bonds to free sulfhydryl groups. The result obtained was considered to be the total thiol. Finally, the disulphide level was considered half the difference between total thioland native thiol. We gathered the 5 mL blood samples of venous into SST tubes (BD Vacutainer SST II Advance, USA). To acquire sera blood tubes were centrifuged 10 min at 1000 × g. Until the assessment was performed, samples were kept at − 20°C. Total thiol, native thiol serum, and disulphide levels were measured by a new automated method defined by Erel and Neselioglu. By using a Cobas c501 chemical analyzer (Roche Diagnostics, Mannheinu Germany), we carried outcome measurements. After measuring total thiols (SH+SS), native (SH), and disulphide levels (SS), native thiol/total thiol percent ratios (SH/SH+SS), disulphide/total thiol percent ratios (SS/SH+SS), and disulphide /native thiol percent ratios (SS/SH) were computed by multiplying with 100. Through the use of Visual Analog Scale (VAS), pain levels at rest and at activity was measured[16]. By utilizing a 0 to 10 visual analogue pain scale, with 0 meaning 'no pain' and 10 meaning 'unbearable pain', pain was assessed. It is a scale where each centimeter is given numerical value with an interval of one centimeter. In the Western Ontario and MacMaster osteoarthritis index (WOMAC), 24 elements isolated into 3 subscales (pain, stiffness, and physical function scales) were combined. The maximum scores that can be obtained from the index are 20 for pain subgroup, 8 for stiffness subgroup, 68 for physical function subgroup. High scores indicate increased pain and stiffness and impaired physical function. So as to assess the functional status, WOMAC, a dependable index for use in Turkish patients who have knee osteoarthritis and standing-up from a chair and 15 meter walking test were used to evaluate the alterations infunctional status[17,18].

Statistical analysis

The SPSS for Windows 18.0 software package (IBM Corporation, Armonk, USA) was employedso as to assess the statistical data. The Kolmogorov-Smirnov test was used to assess the normality of the distributions of variables. Study results were demonstrated as mean±standard deviation. Student's *t*-test was utilized to compare normally distributed numerical variables. The Mann-Whitney U-test was utilized to compare abnormally distributed numerical variables. The chi-square test was utilized to compare the study groups in terms of gender distribution. Pearson correlation test was utilized to evaluate the relationship between normally distributed numerical variables. Spearman correlation test was utilized to evaluate the relationship between abnormally distributed numerical variables. Statistically significant *P* values were less than 0.05.

Results

In Table 1, the parameters of laboratory and the clinical properties of the knee OA patients and control group have been shown. In demographic characteristics of the study groups**,** no significant differences were detected ($P > 0.05$). In the knee OA patients, the mean age was 64.23 ± 8.37 years and 62.10 ± 6.63 years in the control group. In patients with knee OA, it was observed that the disease duration was 5.78 ±3.89 years. When they were compared to control group, in disulphide ($P < 0.05$) and disulphide/total thiol levels ($P < 0.05$) in patients with knee OA, statistically significant differences were observed. In disulphide/native thiol levels of the study groups, no meaningful diffrences were discovered, yet the levels of disulphide/native thiol for the patients with knee were higher than the control

group. When they were compared to patients with knee OA, in native thiol and native/total thiol levels in control group, statistically significant differences were seen ($P<0.05$, respectively). It was found that there were no significant differences in total thiol levels of the study groups, though total thiol levels of the control group were higher than patients with knee OA (Table 2 & Fig. 1A-C).

Activity pain negative was associated with native thiol levels ($r = -0.30$. $P = 0.01$). In patients with knee OA, the disease duration was negatively associated with native thiol and total thiol levels ($r = -0.30$, $P = 0.01$; $r = -0.39$, $P = 0.00$, respectively). A negative relationship between walking test scores and the serum native thiol levels and a positive association between the walking test scores and serum disulphide levels in knee OA patients ($r = -0.37$, $P = 0.00$; $r = 0.33$. $P = 0.00$, respectively). In patients with knee OA, no correlation between serum thiol/ disulphide results and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), WOMAC scores was observed (Table 3).

Table 1 — Demographic characteristics of the study groups

Variables	Patient group (n=60) Mean± SD	Control group (n=50) Mean ± SD
Age (years)	64.23 ± 8.37	62.10 ± 6.63
Gender		
Women, n (%)	% 66.66	% 64
Men, n (%)	% 33.33	% 36
BMI	28.15 ± 2.21	27.43 ± 2.16
Disease duration (years)	5.78 ± 3.89	-
Walking test (seconds)	14.62 ± 3.31	-
VAS (rest pain)	2.91 ± 1.14	-
VAS (activity pain)	6.05 ± 1.34	-
ESR (mm/s)	17.13 ± 11.06	-
CRP (mg/L)	0.5 ± 0.36	-
WOMAC pain	9.55 ± 2.89	-
WOMAC stiffness score	3.46 ± 1.15	-
WOMAC physical function	34.10 ± 8.37	-

SD; standard deviation, BMI; body mass index, ESR; erythrocyte sedimentation rate, CRP; C - reactive protein, *Statistically significant difference ; $P< 0.05$

Table 2 — Plasma thiol-disulphide levels of patient and control group

Variables	Patient group (n=60) Mean± SD	Control group (n=50) Mean ± SD	*P*-values
Native thiol, µ mol/L	332.42 ± 39.68	359.50 ± 96.06	0.04
Total thiol, µ mol/L	382.31 ± 43.42	396.86 ± 94.41	0.28
Native/total thiol, %	87.07 ± 5.98	89.77 ± 6.48	0.02
Disulphide, µ mol/L	24.94 ± 12.47	18.71 ± 7.94	0.00
Disulphide/native thiol, %	7.71 ± 4.27	6.01 ± 4.93	0.05
Disulphide/total thiol, %	6.46 ± 2.99	5.09 ± 3.21	0.02

SD; standard deviation

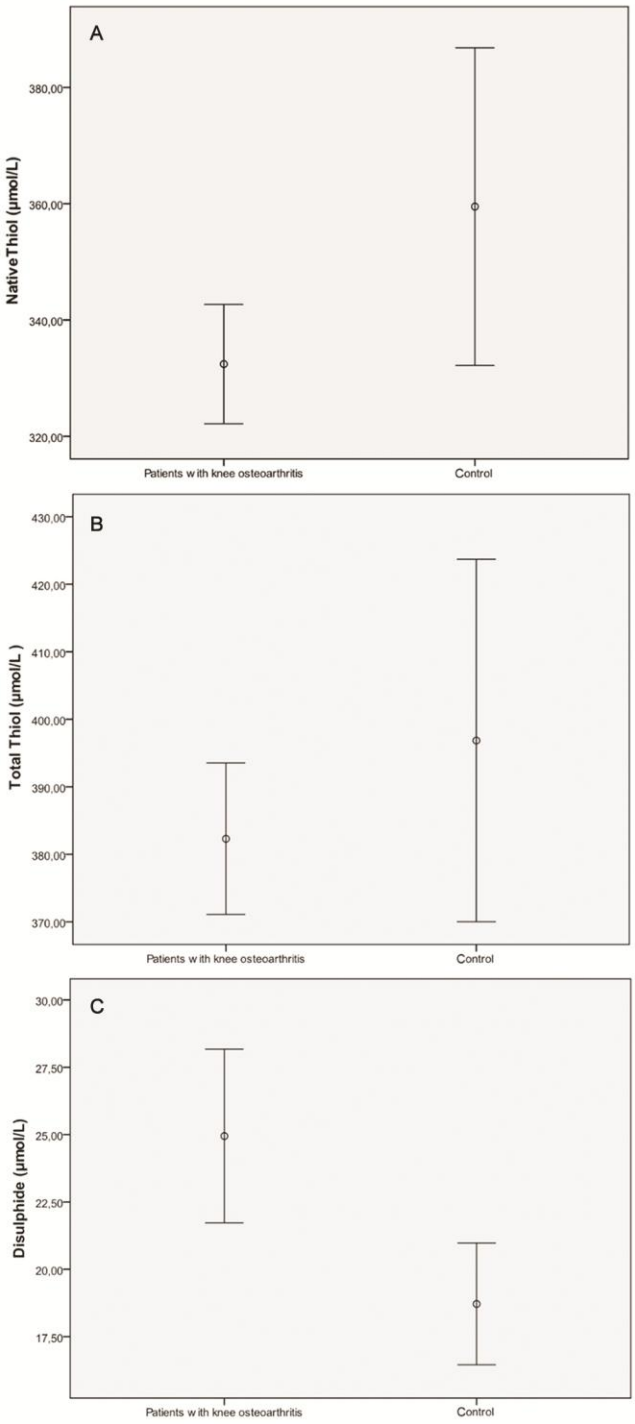

Fig. 1 — (A) Serum native thiol; (B) Serum total thiol; and (C) Serum disulphide levels in patients with knee osteoarthritis and control

Table 3 — The relationship between plasma thiol-disulphide levels and other variables in patient group

Variables	Native thiol	Total thiol	Disulphide	Disulphide/ native thiol	Disulphide/ total thiol	Native/ total thiol
Age (years)						
r	−0.04	−0.03	0.00	−0.10	−0.10	0.10
p	0.74	0.77	0.98	0.26	0.29	0.26
Disease duration (years)						
r	−0.39	−0.30	0.09	0.19	0.19	−0.19
p	0,00*	0.01*	0.49	0.14	0.14	0.14
VAS (rest pain)						
r	−0,15	−0.11	0.04	0.06	0.06	−0.06
p	0.22	0.36	0.73	0.66	0.64	0.64
VAS (activity pain)						
r	−0.30	−0.18	0.15	0.15	0.15	−0.15
p	0.01*	0.14	0.23	0.09	0.09	0.09
Walking test (seconds)						
r	−0.37	−0.14	0.33	0.40	0.40	−0.40
p	0.00*	0.26	0.00*	0.00*	0.00*	0.00*
ESR (mm/s)						
r	−0.16	−0.15	0.20	0.23	0.23	−0.23
p	0.21	0.24	0.12	0.07	0.07	0.07
CRP (mg/L)						
r	0.02	0.00	0.16	0.16	0.16	−0.16
p	0.84	0.97	0.21	0.20	0.20	0.20
WOMAC pain						
r	0.00	0.02	0.03	0.01	−0.01	−0.01
p	0.96	0.84	0.78	0.91	0.90	0.91
WOMAC stiffness score						
r	−0.08	0.03	0.19	0.17	0.17	−0.17
p	0.52	0.79	0.13	0.18	0.18	0.18
WOMAC physical function						
r	−0.12	−0.00	0.18	0.22	0.22	−0.22
p	0.35	0.95	0.16	0.09	0.08	0.09

SD; standard deviation, BMI; body mass index, ESR; erythrocyte sedimentation rate, CRP; C - reactive protein,

*Statistically significant difference ; $P < 0.05$

Discussion

Along with proinflammatory factors like cytokines and prostaglandins, in many of joint diseases, ROS and nitric oxide are released at the inflammation regions[10]. ROS production is displayed to raise in joint diseases like rheumatoid arthritis and osteoarthritis[6]. Moreover, increased oxidative stress in atheroselerosis, diabetes mellitus, neurodegenerative syndromes, and systemic sclerosis were demonstrated in earlier studies[19-21]. It was suggested by Tetik *et al*. that plasma proteins are sensitive to oxidative stress in osteoarthritis, an inflammatory disease and thus oxidative stress increase may be the reason of disease severity. Additionally, it was stated that OA patients are at risk of significant tissue oxidant damage on account of impaired antioxidant defense[22].

OA is a chronic inflammatory disease caused by gradual changes in the immune system[23]. It has been recommended by recent studies that OA development is signifĩcantly linked with oxidative stress and ROS[24,25]. ROS contributes to the loss of chondrocyte sensibility to growth factors[26]. The overproduction of ROS contributes to cartilage destruction, and chondrocytes and collagens are highly sensitive to the cumulative effects of oxidative stress[27]. Oxidative damage has an adverse effect on the collagen[28]. ROS may also attend in the failure of repair by decreasing the capacity of chondrogenic precursor cells to migrate and proliferate within a damaged field[29]. Damage to proteins due to oxidant accumulation causes changes in mechanical damage and the water-holding capacity of collagen fibrils[30]. Oxidative stress has a significant role in chondrocyte apoptosis[31]. Studies show that oxidative stress causes chondrocyte senescence and cartilage ageing[32,33]. Altı ndag *et al*. found that plasma thiol level considerably decreased in OA patients compared to healthy controls. Furthermore, they indicated that decreasing collagen metabolism may be associated with oxidative stress and oxidative stress may have an effect on pathogenesis

and/or disease progression[34]. El-barbary *et al*. suggested that oxidative stress level elevated in OA patients and oxidative stress has a role on the pathogenesis of OA[35]. Soran *et al*. stated that oxidative stress elevated in knee OA patients[36]. In this research, we showed the thiol level, which was known as an significant ingredient of the plasma antioxidant system, was less in knee OA patients than the control group. We found that disulphide/native thiol, disulphide, and disulphide/total thiol ratios were higher in knee OA patients than control group, while total thiol, native thiol, and native/total thiol levels were lower. Since ROS cause disulfide bonds by oxidation of thiol groups of sulfur-containing amino acids in proteins, total thiol levels of the patients with knee OA were lower than the control group. The thiol/disulphide equilibrium was determined to be weaker in knee OA patients than the control group and the balance altered towards disulphide formation. This indicates that the level of oxidative stress is higher in with the knee OA patients than in the control group. The outcomes we obtained were compatible with the previous studies in the literature. We believe that the impairment of plasma antioxidant defense is an important risk factor for tissue oxidant damage in patients with OA. Future studies with large patient populations might help to elucidate the mechanisms of the association of thiol/disulphide homeostasis with OA in the antioxidant defense.

The most widespread chronic diseases causing to physical disablement and pain in patients is OA[37]. OA is frequently related with functional limitation and impaired quality of life[38]. Dogru *et al*. showed a significant negative association between the VAS and the plasma thiol levels in ankylosing spondylitis patients[39]. In this study, we determined a statistically significant negative relationship between the VAS and the plasma native thiol concentrations and disease duration. Moreover, we found a significant relationship between thiol homeostasis and walking test scores in knee OA patients. Nevertheless, no relation was seen between the thiol/disulphide homeostasis outcomes and WOMAC subscales.

There were some limitations in our research. Firstly, the study population was small sample size in our study. Secondly, patients in our study were not followed for a long time. A larger study population with long-term patient follow-up may provide stronger statistical data.

Conclusion

The outcomes of this research demonstrated that in knee OA, oxidative stress is increased, yet thiol/ disulphide homeostasis had not noticeable impact on the on functional status. Thiol/disulfide homeostasis may be an important parameter to elucidate the effects of oxidative stress. We think that the role of thiol/disulphide homeostasis on oxidative stress in OA should be investigated by future studies and this may also help to evaluate the role of oxidative stress on etiopathogenesis of OA.

Conflict of interest

All authors declare no conflict of interest.

References

1 Martel JP & Pelletier JP, Is osteoarthritis a disease involving only cartilage or other articular tissues? *Joint Disease Surgery*, 21 (2010) 2.

2 Gambhir JK, Lali P & Jain AK, Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. *Clin Biochem*, 30 (1997) 351.

3 Xia B, Di C, Zhang J, Hu S, Jin H & Tong P, Osteoarthritis pathogenesis: a review of molecular mechanisms. *Calcif Tissue Int*, 95 (2014) 495.

4 Mazzetti I, Grigolo B, Pulsatelli L Dolzani P, Silvestri T, Roseti L, Meliconi R & Facchini A, Differential roles of nitric oxide and oxygen radicals in chondrocytes affected by osteoarthritis and rheumatoid arthritis. *Clin Sci*, 101 (2001) 593.

5 Ayarl X, Pickering EH, Woodworth TG, Mackillop N & Dougados M, Synovitis: a potential predictive factor of structural progression of medial tibiofemoral Knee osteoarthritis- results of a 1 year longitudinal arthroscopic study in 422 patients. *Osteoarthritis cartilage*, 13 (2005) 361.

6 Deberg M, Labasse A, Christgau S,Cloos P, Bang Henriksen D, Chapelle JP, Zegels B, Reginster JY & Henrotin Y, New serum biochemical markers (Coll 2–1 and Coll 2–1 NO2) for studying oxidative-related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage*, 13 (2005) 258.

7 Sen CK & Packer L, Thiol homeostasis and supplements in physical exercise. *Am J Clin Nutr*, 72 (2000) 653S.

8 Winterbourn CC & Hampton MB, Thiol chemistry and specificity in redox signaling. *Free Radic Biol Med*, 45 (2008) 549.

9 Circu ML & Aw TY, Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med*, 48 (2010) 749.

10 Sakurai H, Kohsaka H, Liu MF, Higashiyama H, Hirata Y, Kanno K, Saito I & Miyasaka N, Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritides. *J Clin Invest*, 96 (1995) 2357.

11 Cremers CM & Jakob U, Oxidant sensing by reversible disulfide bond formation. *J Biol Chem*, 288 (2013) 26489.

12 Biswas S, Chida AS & Rahman I, Redox modifications of protein–thiols: emerging roles in cell signaling. *Biochem Pharmacol*, 71 (2006) 551.

13 Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, Christy W, Cooke TD, Greenwald R & Hochberg M, Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and therapeutic criteria committee of the american rheumatism association. *Arthritis Rheum*, 29 (1986) 1039.

14 Kellgren JH & Lawrence JS, Radiological assessment of osteoarthrosis. *Ann Rheum Dis*, 16 (1957) 494.

15 Erel O & Neselioglu S, A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*, 47 (2014) 326.

16 Wewers ME & Lowe NK, A critical review of visual analogue scales in the measurement of clinical phenomena. *Res Nurs Health*, 3 (1990) 227.

17 Basaran S, Guzel R, Seydaoglu G & Uysal GF, Validity, reliability, and comparison of the WOMAC osteoarthritis index and Lequesne algofunctional index in Turkish patients with hip or knee osteoarthritis. *Clin Rheumatol*, 29 (2010) 749.

18 Gür H, Cakin N, Akova B, Okay E & Kucukoglu S, Concentric versus combined concentric-eccentric isokinetic training: effects on functional capacity and symptoms in patients with osteoarthrosis of the knee. *Arch Phys Med Rehabil*, 83 (2002) 308.

19 Stadtman ER, Protein oxidation in aging and age-related diseases. *Ann N Y Acad Sci*, 928 (2001) 22.

20 Allanore Y, Borderie D, Lemrechal H, Ekindjian OG & Kahan A, Acute and sustained effects of dihydropyridine-type calcium channel antagonists on oxidative stress in systemic sclerosis. *Am J Med*, 116 (2004) 595.

21 Mistry NK, Dabhi KB & Joshi BB, Evaluation of oxidative stress biomarkers and inflammation in pathogenesis of diabetes and diabetic nephropathy. *Indian J Biochem Biophys*, 57 (2020) 45.

22 Tetik S, Ahmad S, Alturfan AA, Fresko I, Disbudak M, Sahin Y, Aksoy H & Yardimci KT, Determination of oxidant stress in plasma of rheumatoid arthritis and primary osteoarthritis patients. *Indian J Biochem Biophys*, 47 (2010) 353.

23 Wojdasiewicz P, Poniatowski LA & Szukiewicz D, The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm*, 2014 (2014) 561459.

24 Li D, Xie G & Wang W, Reactive oxygen species: the 2-edged sword of osteoarthritis. *Am J Med Sci*, 344 (2012) 486.

25 Henrotin Y, Kurz B & Aigner T, Oxygen and reactive oxygen species in cartilage degradation: friends or foes? *Osteoarthritis Cartilage*, 13 (2005) 643.

26 Studer RK, Levicoff E, Georgescu H, Miller L, Jaffurs D & Evans CH, Nitric oxide inhibits chondrocyte response to IGF-I: inhibition of IGF-IRbeta tyrosine phosphorylation. *Am J Physiol Cell Physiol*, 279 (2000) C961.

27 Lepetsos P & Papavassiliou AG, ROS/oxidative stress signaling in osteoarthritis. *Biochimica et Biophysica Acta*, 1862 (2016) 576.

28 Hollander AP, Pidoux I, Reiner A,*Rorabeck C, Bourne R & Poole AR*, Damage to type II collagen in aging and osteo-arthritis starts at the articular surface, originates around chondrocytes, and extends into the cartilage with progressive degeneration. *J Clin Invest*, 96 (1995) 2859.

29 Mateos J, De la Fuente A, Lesende-Rodriguez I, Fernández-Pernas P, Arufe MC & Blanco FJ, Lamin A deregulation in human mesenchymal stem cells promotesan impairment in their chondrogenic potential and imbalance in their response to oxidative stres. *Stem Cell Res*, 11 (2013) 1137.

30 Regan E, Flannelly J, Bowler R, Tran K, Nicks M, Carbone BD, Glueck D, Heijnen H, Mason R & Crapo J, Extracellular Superoxide Dismutase and Oxidant Damage in Osteoarthritis. *Arthritis Rheum*, 52 (2005) 3479.

31 Blanco FJ, Guitian R, Vazquez-Martul E, de Toro FJ & Galdo F, Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. *Arthritis Rheum*, 41 (1998) 284.

32 Yudoh K, Nguyen T, Nakamura H, Hongo-Masuko K, Kato T & Nishioka K, Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. *Arthritis Res Ther*, 7 (2005) 380.

33 Martin JA, Brown TD, Heiner AD & Buckwalter JA, Chondrocyte senescence, joint loading and osteoarthritis. *Clin Orthop Relat Res*, 427 (2004) S96.

34 Altindag O, Erel O, Aksoy N, Selek S, Celik H & Karaoglanoglu M, Increased oxidative stress and its relation with collagenmetabolism in knee osteoarthritis. *Rheumatol Int*, 27 (2007) 339.

35 El-barbary AM, Khalek MAA, Elsalawy AM & Hazaa SM, Assessment of lipid peroxidation and antioxidant status in rheumatoid arthritis and osteoarthritis patients. *Egypt Rheumatol*, 33 (2011) 179.

36 Soran N, Altindag O, Cakir H, Celik H, Demirkol A & Aksoy N, Assesment of paraoxonase activities in patients with knee osteoarthritis. *Redox Rep*, 13 (2008) 194.

37 Henrotin Y & Kurz B, Antioxidant to treat osteoarthritis: dream or reality? *Curr Drug Targets*, 8 (2007) 347.

38 Towheed TE, Maxwell L, Anastassiades TP, Shea B, Houpt J, Robinson V, Hochberg MC & Wells G, Glucosamine therapy for treating osteoarthritis. *Cochrane Database Syst Rev*, 18 (2005) CD002946.

39 Dogru A, Balkarli A, Cetin GY, Neselioglu S, Erel O, Tunc SE & Sahin M, Thiol/disulfide homeostasis in patients with ankylosing spondylitis. *Bosn J Basic Med Sci*, 16 (2016) 187.