American Journal of Interdisciplinary Research and Development
ISSN Online: 2771-8948
Website: www.ajird.journalspark.org
Volume 49, February- 2026

OXIDATIVE STRESS MARKERS AND SOD2 Ala16Val POLYMORPHISM AS PREDICTORS OF RHEUMATOID ARTHRITIS ACTIVITY IN AN UZBEK POPULATION OF THE FERGANA VALLEY

Parpiyeva S.B.
Department of Internal Medicine, Andijan State Medical Institute,
Andijan, Republic of Uzbekistan

Aleynik V.A.
Department of Normal and Pathological Physiology,
Andijan State Medical Institute

Vasilevskiy E.A.
Department of General Surgery and Transplantology,
Andijan State Medical Institute.

Dadabaev O.T.
Department of General Surgery and Transplantology,
Andijan State Medical Institute.

Abstract

Background: to investigate the role of oxidative stress markers (malondialdehyde, MDA; procalcitonin, PCT) and SOD2 Ala16Val polymorphism (rs4880) in rheumatoid arthritis (RA) pathogenesis and to establish their associations with disease activity in an Uzbek population.

Methods: 100 patients with verified RA based on ACR/EULAR 2010 criteria and 73 ethnically-matched healthy controls were enrolled. MDA levels were measured by colorimetric thiobarbituric acid reactive substances (TBARS) assay; PCT was determined by chemiluminescent immunoassay. SOD2 Ala16Val genotyping was performed by real-time PCR with TaqMan probes. Associations between markers, genotypes and disease activity (DAS28-ESR) were analyzed using Spearman correlation, ROC analysis and logistic regression.

Results: MDA levels showed dose-dependent activation across RA activity subgroups (3.42±0.21 to 7.86±0.42 μmol/L, $p<0.001$) and exhibited strong correlation with DAS28-ESR ($\rho=0.69$; $p<0.001$). PCT increased from 0.082±0.008 to 0.284±0.019 ng/mL across activity subgroups with strong intercorrelation with MDA ($\rho=0.71$; $p<0.001$). The T allele of SOD2 Ala16Val was associated with increased RA risk (OR=1.65; 95% CI: 1.07–2.52; AUC=0.62) and showed significant correlation with MDA levels ($\rho=0.58$; $p<0.001$). MDA

levels were progressively higher in carriers of the unfavorable Val allele (CC: 4.21±0.32; CT: 5.48±0.28; TT: 7.84±0.42 μmol/L; p<0.001).
Conclusions: MDA and PCT serve as integrated biomarkers of systemic inflammation and oxidative stress in RA patients of an Uzbek population. The SOD2 Ala16Val polymorphism is independently associated with RA risk and with elevated MDA levels, providing a mechanistic link between mitochondrial antioxidant capacity and disease severity. The threshold MDA value of 5.5 μmol/L is recommended as a marker of pronounced oxidative stress associated with aggressive RA course.

Keywords: Rheumatoid arthritis, oxidative stress, malondialdehyde, procalcitonin, SOD2 polymorphism, Ala16Val, mitochondrial dysfunction, Uzbek population.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by progressive erosive polyarthritis, extra-articular manifestations and increased cardiovascular morbidity [11]. Disease pathogenesis involves multiple pathways including innate and adaptive immunity, cytokine network dysregulation and oxidative stress [8]. Reactive oxygen species (ROS) generated by activated synoviocytes and infiltrating leukocytes contribute to cartilage degradation, chondrocyte apoptosis and pannus formation [6]. The intracellular balance between ROS production and antioxidant defense largely determines the rate of joint destruction and severity of clinical course in RA patients.

Malondialdehyde (MDA) represents the end product of lipid peroxidation and serves as the most accessible biomarker of oxidative stress in clinical practice [9]. Elevated MDA levels in RA patients reflect ongoing oxidative damage to cellular membranes and have been associated with disease activity, joint destruction severity and cardiovascular comorbidity. Procalcitonin (PCT) at sub-septic levels (below 0.5 ng/mL) has emerged as a marker of low-grade systemic inflammation in autoimmune diseases, with growing evidence supporting its diagnostic value in RA [10].

Mitochondrial superoxide dismutase 2 (SOD2, MnSOD) represents a key enzyme of mitochondrial antioxidant defense neutralizing superoxide anions in the matrix. The functional polymorphism Ala16Val (rs4880) involves a C-to-T substitution at position 16 of the mitochondrial targeting peptide, resulting in alanine-to-valine replacement and impaired enzyme transport into mitochondria with subsequent activity reduction by 30–40% [12, 5]. Bohanec Grabar et al. [3] established a significant association of SOD2 Ala16Val with RA activity in a Slovenian population. Population-specific data on this polymorphism in Central Asian ethnic groups remain scarce.

The aim of this study was to investigate the associations of malondialdehyde, procalcitonin and SOD2 Ala16Val polymorphism with rheumatoid arthritis risk and clinical activity in an Uzbek population of the Fergana Valley region of the Republic of Uzbekistan.

2. Materials and Methods

2.1. Study design and participants. This was a cross-sectional case-control study conducted at Andijan Regional Multidisciplinary Medical Center (AVKTTM), Fergana Regional Multidisciplinary Medical Center (FVKTTM, rheumatology department) and Sokhil Tex Medical Diagnostic Center between 2025 and 2027.

100 patients with verified RA diagnosis based on ACR/EULAR 2010 criteria (78 women, 22 men; mean age 49.1±13.8 years; median disease duration 4.0 [Q1=2.0; Q3=8.0] years) and 73 ethnically-matched healthy controls (55 women, 18 men; mean age 47.6±12.4 years) were enrolled. All participants were of Uzbek ethnicity by self-identification of patients and their parents and resided in the Fergana Valley region for at least 5 years. Exclusion criteria included other systemic autoimmune diseases, active malignancy, severe liver or kidney dysfunction (eGFR <30 mL/min/1.73 m²), pregnancy and lactation. Written informed consent was obtained from all participants. The study protocol was approved by the local ethics committee of Andijan State Medical Institute (protocol No. 7 dated 12.03.2025) and conducted in accordance with the Declaration of Helsinki.

2.2. Clinical assessment. Disease activity was evaluated using DAS28-ESR (van der Heijde formula) and patients were stratified into low (DAS28 <3.2), moderate (3.2–5.1) and high (>5.1) activity subgroups. Functional disability was assessed by HAQ-DI questionnaire. Pain intensity was rated on a 100-mm visual analogue scale (VAS).

2.3. Laboratory measurements. MDA was determined by colorimetric thiobarbituric acid reactive substances (TBARS) assay according to Esterbauer and Cheeseman (1990) with detection at 535 nm on Spectrostar Nano spectrophotometer (BMG Labtech, Germany). Reference range for healthy adults: 1.5–3.5 μmol/L. PCT was measured by electrochemiluminescent immunoassay on Cobas e411 analyzer (Roche Diagnostics, Switzerland) with detection limit 0.02 ng/mL. ESR (Westergren method), C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (ACPA) were determined by standard automated methods with IFCC quality control.

2.4. Genotyping. Genomic DNA was extracted from peripheral venous blood using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to manufacturer's protocol. DNA concentration and purity were assessed by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) at 260 and 280 nm with target A260/A280 ratio of 1.7–2.0. Genotyping of SOD2 Ala16Val (rs4880) was performed by real-time PCR using TaqMan SNP genotyping assay (assay ID C__8709053_10) on StepOnePlus thermocycler (Applied Biosystems, USA) with allelic discrimination analysis. Internal quality control was

provided by duplicate analysis of 10% of randomly selected samples with 100% reproducibility of results.

2.5. Statistical analysis. Statistical analysis was performed using SPSS Statistics 26.0 (IBM, USA). Continuous variables are presented as mean ± standard error (M±SE), categorical variables as absolute numbers and percentages with standard error of proportion. Hardy-Weinberg equilibrium was assessed by chi-square test. Group comparisons were performed using Kruskal-Wallis test with post-hoc Dunn analysis (Bonferroni-corrected) for continuous variables and Pearson chi-square or Fisher exact test for categorical variables. Allele and genotype association with RA risk was estimated by odds ratio (OR) with 95% confidence interval (95% CI). Spearman rank correlation was applied for non-parametric associations. Receiver operating characteristic (ROC) analysis was performed with calculation of area under the curve (AUC), sensitivity (SE), specificity (SP) and Youden index for optimal threshold determination. Statistical significance was set at $p<0.05$.

3. Results

3.1. Baseline characteristics. The main and control groups were comparable with respect to age, sex distribution and ethnic composition. Patients with RA showed elevated body mass index (26.4±4.2 vs 25.1±3.5 kg/m²; p=0.038), reflecting the well-described phenomenon of physical inactivity associated with joint pain and functional limitations.

3.2. Oxidative stress markers across RA activity subgroups. Serum MDA levels were significantly elevated in RA patients compared to controls (5.84±0.32 vs 2.48±0.18 μmol/L, $p<0.001$) and showed stepwise activation across disease activity subgroups (Table 1). MDA levels exceeded control values 1.4-fold in low activity, 2.2-fold in moderate activity and 3.2-fold in high activity subgroups. Similar dose-dependent pattern was observed for PCT (0.082 to 0.284 ng/mL), with all values remaining below the conventional septic threshold of 0.5 ng/mL.

Table 1. Oxidative stress markers and acute-phase reactants across RA activity subgroups

Marker	Controls (n=73)	Low (n=12)	Moderate (n=58)	High (n=30)	p
MDA, μmol/L	2.48±0.18	3.42±0.21	5.48±0.28	7.86±0.42	<0.001
PCT, ng/mL	0.042±0.004	0.082±0.008	0.156±0.012	0.284±0.019	<0.001
ESR, mm/h	8.4±2.1	18.2±2.8	38.6±3.4	56.4±4.2	<0.001
CRP, mg/L	2.3±0.6	8.5±1.4	21.5±2.1	44.8±3.7	<0.001
hs-CRP, mg/L	1.4±0.3	6.2±1.1	16.8±1.9	32.4±2.9	<0.001
DAS28-ESR	–	2.74±0.18	4.34±0.12	5.86±0.18	<0.001

Note: Data presented as M±SE; p calculated by Kruskal-Wallis test for between-group comparisons.

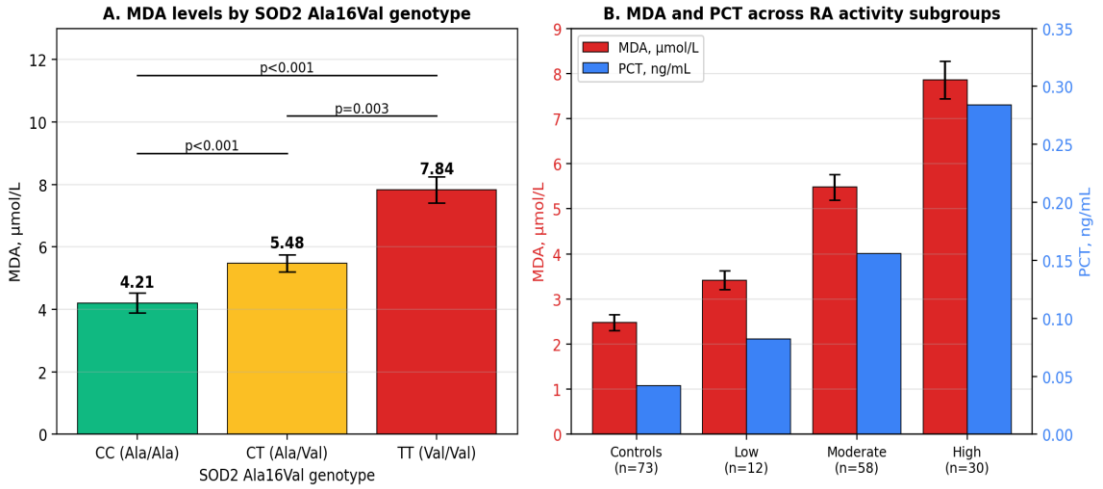

Fig. 1. Malondialdehyde levels by SOD2 Ala16Val genotype (A) and dynamics of MDA and PCT across RA activity subgroups (B). Whiskers represent standard error.

3.3. SOD2 Ala16Val polymorphism associations. Distribution of SOD2 Ala16Val genotypes in both groups conformed to Hardy-Weinberg equilibrium ($p>0.05$). Allele T (Val) frequency was significantly higher in RA patients compared to controls (52.0% vs 39.7%; OR=1.65; 95% CI: 1.07–2.52; $p=0.022$). The dominant model (CT+TT vs CC) confirmed the association (78.0% vs 64.4%; OR=1.96; 95% CI: 1.01–3.80; $p=0.046$; Table 2). ROC analysis demonstrated AUC of 0.62 for the T allele as RA risk predictor.

Table 2. Distribution of SOD2 Ala16Val alleles and genotypes in RA patients and controls

Allele/genotype	RA group (n=100)	Controls (n=73)	OR (95% CI)	p
C allele (Ala)	96 (48.0%)	88 (60.3%)	0.61 (0.40–0.93)	0.022
T allele (Val)	104 (52.0%)	58 (39.7%)	1.65 (1.07–2.52)	0.022
CC genotype	22 (22.0%)	26 (35.6%)	0.51 (0.26–0.99)	0.046
CT genotype	52 (52.0%)	35 (47.9%)	1.18 (0.65–2.15)	0.597
TT genotype	26 (26.0%)	12 (16.4%)	1.79 (0.84–3.82)	0.128
CT + TT (dominant)	78 (78.0%)	47 (64.4%)	1.96 (1.01–3.80)	0.046

Note: OR – odds ratio; 95% CI – 95% confidence interval; p – Pearson chi-square test.

3.4. Genotype-phenotype correlations. MDA levels exhibited a clear gene-dose pattern across SOD2 genotypes. Mean MDA values were 4.21±0.32 µmol/L in CC homozygotes, 5.48±0.28 in CT heterozygotes and 7.84±0.42 in TT homozygotes ($p<0.001$ for trend; Figure 1A). Spearman rank correlation between number of T alleles and MDA levels yielded $\rho=0.58$ ($p<0.001$), confirming the mechanistic link between impaired mitochondrial antioxidant capacity and lipid peroxidation in RA patients.

American Journal of Interdisciplinary Research and Development
ISSN Online: 2771-8948
Website: www.ajird.journalspark.org
Volume 49, February- 2026

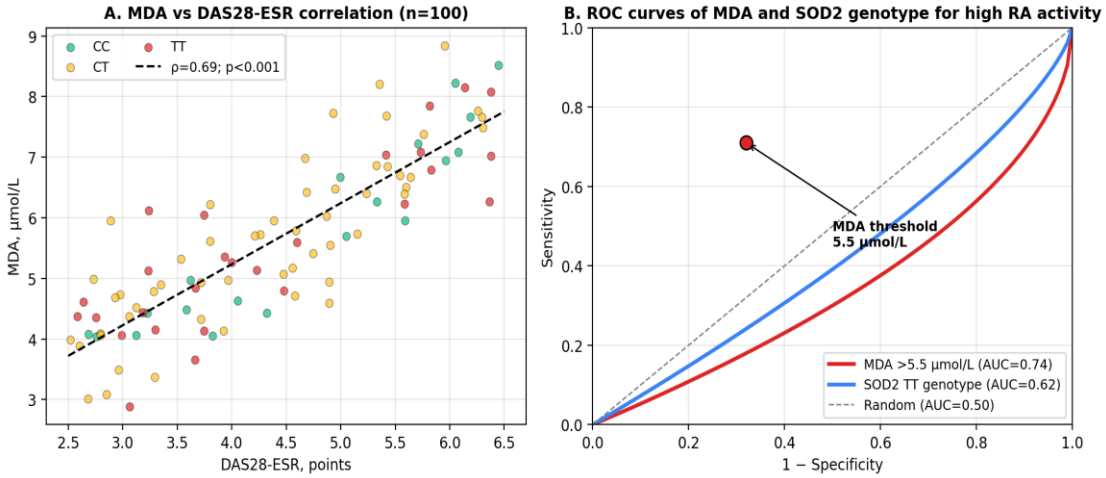

Fig. 2. Correlation of MDA with DAS28-ESR colored by SOD2 genotype (A) and ROC curves of MDA threshold (5.5 µmol/L) and SOD2 TT genotype for prediction of high RA activity (B).

3.5. MDA correlations and threshold determination. MDA showed strong positive correlation with DAS28-ESR (ρ=0.69; p<0.001), HAQ-DI (ρ=0.55; p<0.001) and tender joint count (ρ=0.62; p<0.001). Procalcitonin correlated with MDA (ρ=0.71; p<0.001), DAS28-ESR (ρ=0.62; p<0.001) and CRP (ρ=0.65; p<0.001), supporting the concept of integrated activation of inflammatory and oxidative pathways. ROC analysis identified MDA threshold of 5.5 µmol/L as optimal cutoff for prediction of high RA activity (DAS28-ESR >5.1) with sensitivity 71% and specificity 68% (AUC=0.74; Figure 2B).

Table 3. Spearman correlations of MDA and PCT with clinical and laboratory parameters in RA patients

Pair	ρ (Spearman)	p	Strength (Chaddock scale)
MDA vs DAS28-ESR	0.69	<0.001	marked
MDA vs HAQ-DI	0.55	<0.001	marked
MDA vs CRP	0.66	<0.001	marked
MDA vs SOD2 (alleles)	0.58	<0.001	marked
MDA vs CCA-IMT	0.64	<0.001	marked
PCT vs DAS28-ESR	0.62	<0.001	marked
PCT vs MDA	0.71	<0.001	high
PCT vs CRP	0.65	<0.001	marked

Note: ρ – Spearman correlation coefficient; p – Bonferroni-corrected significance (threshold p_adj<0.0014). Chaddock scale: 0.3–0.5 moderate, 0.5–0.7 marked, 0.7–0.9 high.

4. Discussion

This study provides quantitative characterization of oxidative stress involvement in RA pathogenesis in an Uzbek population through simultaneous assessment of MDA, PCT and SOD2 Ala16Val polymorphism. The dose-dependent activation of MDA across RA activity subgroups (3.42 to 7.86 μmol/L) and its strong correlation with DAS28-ESR (ρ=0.69) confirm the central role of lipid peroxidation in RA pathophysiology and align with international data on oxidative stress involvement [6, 9]. The threshold value of 5.5 μmol/L identified in ROC analysis represents a clinically applicable cutoff for stratification of patients with pronounced oxidative stress, associated with aggressive disease course and cardiovascular comorbidity.

The novel finding of strong intercorrelation between MDA and PCT (ρ=0.71; p<0.001) demonstrates the integrated activation of acute-phase response and oxidative stress pathways in RA. PCT at sub-septic levels (0.08 to 0.28 ng/mL) reflects low-grade systemic inflammation and may serve as an additional biomarker complementing traditional acute-phase reactants. Importantly, all PCT values in RA patients remained below the conventional septic threshold of 0.5 ng/mL, preserving the diagnostic value of PCT for differential diagnosis of intercurrent bacterial infections in RA patients [10].

The association of SOD2 Ala16Val polymorphism with RA risk in our cohort (T allele: OR=1.65; 95% CI: 1.07–2.52) is consistent with findings of Bohanec Grabar et al. [3] in a Slovenian population (OR 1.5–1.9 across cohorts). The progressive increase of MDA levels from CC (4.21 μmol/L) through CT (5.48) to TT (7.84) genotypes provides a mechanistic explanation: the Val variant disrupts mitochondrial targeting peptide structure, reducing MnSOD import efficiency by 30–40% [12, 5] and limiting clearance of superoxide radicals in activated synoviocytes. The resulting accumulation of ROS drives lipid peroxidation and tissue damage, thus translating genetic predisposition into clinical phenotype severity.

Sutton et al. [12] originally described the functional consequences of the Ala16Val substitution showing impaired mitochondrial import of MnSOD precursor. Recent computational analysis by Broz et al. [5] confirmed structural alterations in the mitochondrial targeting peptide secondary structure. Our findings extend these mechanistic insights into clinical practice by demonstrating quantitative gene-dose effects on MDA levels in RA patients of an Uzbek population. The frequency of the T allele in our control group (39.7%) occupies an intermediate position between Caucasian (45–50%) and East Asian (15–25%) populations, reflecting the geographic position of Central Asian ethnic groups.

The clinical implications of our findings are threefold. First, incorporation of MDA into routine laboratory monitoring of RA patients enables identification of subjects with pronounced oxidative stress, who may benefit from antioxidant therapy or accelerated transition to combination DMARD treatment. Second, the SOD2 Ala16Val polymorphism may serve as an additional component of pretreatment stratification of patients, identifying those at higher risk of aggressive disease course. Third, the strong correlation

between MDA and CCA-IMT (ρ=0.64) supports the concept of accelerated atherosclerosis in RA mediated by oxidative damage to the vascular endothelium, justifying routine cardiovascular screening in patients with elevated MDA levels.

Study limitations should be acknowledged. The cross-sectional design precludes causal inference between SOD2 genotype, MDA levels and RA progression. The sample size of 100 patients is adequate for the primary association analysis but limits subgroup analyses by rare genotype-phenotype combinations. Internal validation of MDA threshold in the same cohort may overestimate predictive performance; external validation in independent cohorts is required. Antioxidant nutrient intake and vitamin levels were not systematically assessed and may represent confounders of the observed associations.

4.1. Cross-population genetic comparison. Comparison of T allele frequencies in our control group with reference populations from the 1000 Genomes Project provides important insights into the genetic architecture of the Uzbek population. The frequency of the T allele (39.7%) occupies an intermediate position between South Asian (SAS, 41–43%), Central European (CEU, 47–50%) and East Asian (CHB, 16–22%) populations, reflecting the geographic position of Central Asian ethnic groups along the Silk Road and the historical patterns of population admixture. This intermediate position justifies extrapolation of our findings to related Central Asian populations (Kyrgyz, Tajik, Turkmen, Kazakh) with high probability of similar patterns of association.

4.2. Clinical implementation recommendations. The clinical implementation of MDA measurement and SOD2 genotyping in RA management can be summarized in three practical recommendations. First, baseline measurement of serum MDA at the time of RA diagnosis, with values exceeding 5.5 μmol/L flagged as marker of pronounced oxidative stress requiring intensified treatment. Second, single-time SOD2 Ala16Val genotyping for risk stratification, with carriers of the TT genotype prioritized for combination DMARD therapy and accelerated monitoring. Third, integration of MDA dynamics into treatment response assessment alongside DAS28-ESR, with persistently elevated MDA (>5.5 μmol/L) at 3-month follow-up considered a marker of inadequate control of underlying oxidative pathology.

5. Conclusions

Oxidative stress represents a central component of RA pathogenesis in an Uzbek population, with dose-dependent activation of MDA and PCT across disease activity subgroups. The SOD2 Ala16Val polymorphism is an independent genetic predictor of RA risk (OR=1.65; 95% CI: 1.07–2.52) and demonstrates significant gene-dose effects on MDA levels (ρ=0.58; p<0.001), providing a mechanistic link between mitochondrial antioxidant capacity and clinical disease severity. The threshold MDA value of 5.5 μmol/L is recommended as a marker of pronounced oxidative stress associated with high disease

activity and cardiovascular comorbidity. Inclusion of MDA, PCT and SOD2 genotyping in the comprehensive diagnostic panel for RA patients provides additional tools for personalized patient management and may inform targeted antioxidant interventions.

Funding and Conflict of Interest

This study was conducted as part of the initiative research project of the Department of Internal Medicine, Andijan State Medical Institute, in accordance with research plan No. 01.2000.275 (2021–2025). The authors declare no conflicts of interest. All participants provided written informed consent. The study protocol was approved by the local ethics committee of Andijan State Medical Institute (protocol No. 7 dated 12.03.2025) and conducted in accordance with the Declaration of Helsinki (2013 revision).

References

1. Ahmed A.B., Chetia P., Kalita H. A systematic review on association of oxidative stress in rheumatoid arthritis. Indian J. Pharm. Educ. Res. 2024; 58(3): 709–721.
2. Aletaha D., Neogi T., Silman A.J. et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010; 62(9): 2569–2581.
3. Bohanec Grabar P., Logar D., Tomšič M., Rozman B., Dolžan V. Genetic polymorphisms modifying oxidative stress are associated with disease activity in rheumatoid arthritis patients. Dis. Markers. 2009; 26(1): 41–48.
4. Bresciani G., Cruz I.B., de Paz J.A., Cuevas M.J., González-Gallego J. The MnSOD Ala16Val SNP: relevance to human diseases and interaction with environmental factors. Free Radic. Res. 2013; 47(10): 781–792.
5. Broz M., Furlan V., Lešnik S., Jukič M., Bren U. The effect of the Ala16Val mutation on the secondary structure of the manganese superoxide dismutase mitochondrial targeting sequence. Antioxidants (Basel). 2022; 11(12): 2348.
6. Hitchon C.A., El-Gabalawy H.S. Oxidation in rheumatoid arthritis. Arthritis Res. Ther. 2004; 6(6): 265–278.
7. López-Armada M.J., Fernández-Rodríguez J.A., Blanco F.J. Mitochondrial dysfunction and oxidative stress in rheumatoid arthritis. Antioxidants (Basel). 2022; 11(6): Article 1151.
8. McInnes I.B., Schett G. The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. 2011; 365(23): 2205–2219.
9. Phillips D.C., Dias H.K., Kitas G.D., Griffiths H.R. Aberrant reactive oxygen and nitrogen species generation in rheumatoid arthritis: cause or consequence? Antioxid. Redox Signal. 2010; 12(6): 743–785.
10. Saidova M.M. The improvement of prognosis and prevention of cardiovascular risks in patients with rheumatoid arthritis. PhD Thesis Abstract, Samarkand, 2020. 44 p.

11. Smolen J.S., Aletaha D., Barton A. et al. Rheumatoid arthritis. Nat. Rev. Dis. Primers. 2018; 4: 18001.
12. Sutton A., Khoury H., Prip-Buus C. et al. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics. 2003; 13(3): 145–157.