The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2023

Volume 10, Pages 49-54

ICMeHeLS 2023: International Conference on Medical, Health and Life Sciences

Redox State and Biogenic Elements in Osteoporosis with Different Localization

Radka Tomova
Medical University of Pleven

Svetla Asenova
Medical University of Pleven

Lilia Atanasova
Medical University of Sofia

Rodina Nestorova
Rheumatology Centre St. Irina

Bisera Atanasova
Medical University of Sofia

Tzvetanka Petranova
UMedical University of Sofia

Mariana Nikolova
Middlesex University

Miglena Slavova
Institute of Electrochemistry and Energy Systems

Radka Tafrajijska-Hadjiolova
Medical University of Sofia

Abstract: The redox state of the human body is the result of a complex influence of enzymes, metabolites, biogenic elements, and endogenous lifestyle factors. With age, unfavorable changes occur and degenerative and pathogenic processes are unlocked, various diseases appear, such as osteoporosis. In the present work, the radical scavenging activity (RSA%) and the blood serum conclusions of copper and magnesium were investigated in patients with osteoporosis of the hip, as well as in patients with osteoporosis of the lumbar spine. 66 menopausal and postmenopausal women aged 63.41±7.81 were investigated - newly diagnosed with osteoporosis and controls. Exclusion criteria were as follows: diabetes, endocrine and liver disease; intake of estrogenic and biogenic elements. Bone mineral density (BMD) was measured in all patients using dual-energy X-ray absorptiometry (DEXA). Patients with T-Score ≤ -2.5 were divided into two groups – with osteoporosis of the hip and with osteoporosis of the lumbar spine. A control group with T-Score > -1.0 was formed. Serum RSA% was established using the experimental method for determining antioxidants ABTS decolonization assay. Serum copper and magnesium levels were determined using atomic absorption analysis. According to the RSA% indicator, we obtained the following values: 73.30 ± 9.84 in the group with lumbar vertebrae osteoporosis; 68.87 ± 13.26 in the hip osteoporosis group; 55.67 ± 1.37 in the control group. When monitoring the radical scavenging activity, depending on the localization of the disease, we detected higher values in patients with osteoporosis of the lumbar spine. Serum copper and magnesium concentrations of all patients

were elevated relative to the controls, but there was no statistical difference between groups with different disease localization. Patients with osteoporosis were found to have a higher protective antioxidant capacity in response to increased free-radical processes in the body and cellular metabolic disorders.

Keywords: Osteoporosis, Hip, Lumbar spine, RSA, Bioelements.

Introduction

The redox state in living organisms is the balance between the opposing functions of oxidizing agents and reducing agents in them. It is determined by the type and quantitative ratio of active oxidants, such as reactive oxygen species (ROS) and nitrogen species (RNS), and active enzymatic and non-enzymatic antioxidants. (Lazarova et al., 2019) ROS are free radicals, products of cellular metabolism that are primarily generated in the mitochondria. They are necessary for the human body as signaling substances for cells. Simultaneously with this process, there are also biochemical processes for trapping free radicals by antioxidants. With a reduced concentration of antioxidants in the body and increased production of ROS, secondary chain-radical processes begin with lipids, proteins, and DNA molecules, resulting in more aggressive oxygen species. The systemic imbalance between oxidizers and reducers, the so-called oxidative stress leads to irreversible changes and loss of functions of molecules, cells, and organs. (Schieber et al., 2014). It is the cause of the appearance of various diseases, one of which is osteoporosis.

Oxidative stress affects a significant proportion of menopausal women (Sakac et al., 2000). Oxidative stress impairs bone remodeling and lowers bone density (Asenova et al., 2020). The antioxidant activity of women with osteoporosis is increased. This is believed to be a response of the bone marrow stem cells, which respond to the higher concentration of oxidants by increasing their radical scavenging activity (RSA%). This explains the higher antioxidant activity of the patients compared to that of the control group of women without osteoporosis (Romάn et al., 2017). The reason for this is the fact that ROS in high concentration, which exists in a deteriorated redox state, also attacks enzymes-metalloproteins. In the oxidative destruction of Cu, Zn-SOD, the protein is degraded and copper ions are released (Kwon et al., 2000; Valko et al., 2016). Our previous study confirmed an increased serum concentration of the trace elements copper and zinc, with the Cu/Zn molar ratio corresponding to the degree of the disease. (Tomova et al., 2020). The released copper ions are redox active and participate in secondary radical reactions and increase antioxidant activity in patients with osteoporosis (Tomova et al., 2022). The increase of radicals in the body impairs bone homeostasis and decreases bone mineral density (BMD) (de Romaña et al., 2016; Valko et al., 2005). Conversely, BMD is positively affected by dietary antioxidant supplementation in postmenopausal women (X. Wu et al., 2017; Rondanelli et al., 2021; Mazzanti et al., 2015).

Results published on micronutrient concentrations in menopausal and postmenopausal women to this date are conflicting and do not explain their role in bone homeostasis. Published studies to determine the level of radical scavenging capacity in patients with osteoporosis and osteopenia are few and do not demonstrate synergistic relationships with the level of essential elements, BMD, and anthropometric indicators.

Osteoporosis depends on many factors and requires multivariate statistical analysis to study the relationships between the different clinical indicators characteristic of this disease. Therefore, in our study, we applied multivariate statistical analysis to the obtained data from 59 patients. The aim was to clarify the relationship between bone density, the level of biogenic elements copper, zinc, magnesium, iron, and calcium, and the level of antioxidant activity (AOA) in newly diagnosed patients with osteopenia and osteoporosis. The results were surprising. After the clustering of the data, not two, but three clusters were formed describing three different stages of bone metabolism disturbance with a different interval in the variations of the studied indicators.

The cluster of patients with the lowest level of BMD had the highest levels of Mg and Cu, and the lowest levels of Zn, Fe, AOA, and BMI. The factor analysis and principal component analysis performed grouped Mg and Cu as well as Ca as discriminating parameters related to the state of reduced bone density. They determine over 70% of the total variation of the system (Tomova et al., 2022). 3D-plot of factor loadings reveals that AOA is a hidden factor related to the degree of bone density reduction. But bone density and degree of disease (osteopenia or osteoporosis) do not fall into the same cluster, which can be explained by the large difference in bone density in different places of localization of the disease (Tomova et al., 2022). This determined the division of patients according to disease location in our subsequent studies.In the present work, the radical scavenging activity (RSA%), the concentrations of copper and magnesium in blood serum of patients with osteoporosis of the hip, as well as of patients with osteoporosis of the lumbar spine, were investigated.

Materials and Methods

66 menopausal and postmenopausal women aged 63.41 ± 7.81 years - newly diagnosed with osteoporosis and controls - were studied. The exclusion criteria were as follows: diabetes, endocrine and liver diseases; intake of estrogens, and biogenic elements. The bone mineral density (BMD) of all participants was measured using dual-energy X-ray absorptiometry (DEXA).

Patients with t-Score ≤-2.5 are divided into two groups – with osteoporosis of the hip and with osteoporosis of the lumbar vertebrae. A control group with t-score >-1.0 was also formed. The bone mineral density (BMD) of all participants was measured using dual-energy X-ray absorptiometry (DEXA). Patients with t-Score ≤-2.5 are divided into two groups – with osteoporosis of the hip and with osteoporosis of the lumbar vertebrae. A control group with t-Score >-1.0 was also formed.

The body mass index (BMI) of all participants was measured. Venous blood was drawn from all controls and patients by a standard procedure following quality assurance requirements in the pre-analytical phase. After centrifugation of the blood at room temperature for 10 min, serum was separated for analysis of RSA%, copper, and magnesium. The serum was frozen if not analyzed immediately. Determination of RSA% is performed within 14 days of sample collection.

Methods of Analysis

Spectrophotometric ABTS-test for Determination of RSA% in Serum

To determine the redox status of the patients, blood serum was examined using the experimental spectrophotometric method for the determination of antioxidants, ABTS-test (Re et al., 1999). The stable green radical cation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) was added to the samples. The staining intensity of the samples decreases after interaction with the antioxidants from the blood serum. We measured the absorbance of the serum samples at 734 nm. The change in absorbance is an indicator of RSA, i.e. the intensity of the ongoing processes and their impact on the patient's condition. A calibration curve RSA%/Trolox, μmol/L was constructed, according to which RSA% was recalculated into Trolox equivalent (TE) - antioxidant capacity, per μL of serum. The calibration curve has a very good degree of linearity.

Flame Atomic Absorption Analysis of Copper and Magnesium in Serum

Blood serum copper and magnesium levels were determined by flame atomic absorption analysis (AAnalyst, Perkin Elmer). For magnesium analysis, serum was diluted 1:50 with 0.25% LaCl3 solution before quantitative analysis. For copper analysis, serum was diluted 1:3 with bidistilled water before quantitative analysis.

Statistical Analysis:

The obtained values for the studied indicators and anthropometric data are presented as mean values ± SD for the respective groups. The statistical significance of all data was assessed by analysis of variance with an unpaired t-test. The statistically significant difference between groups: patients with osteoporosis of the hip; patients with osteoporosis of the lumbar vertebrae and a control group we determined at $p < 0.05$. From the obtained degree of linearity of the calibration curve RSA%/Trolox, μmol/L the coefficient of determination was calculated to be 99.83. The univariate regression analysis performed showed that 99.83% of the changes in RSA% value were due to the changes in Trolox concentration.

Results and Discussion

Results

The evaluation was done by the analysis of variance with unpaired Student's t-test between different groups with $P < 0.05$ for statistical significance (Table 1). The results of Table 1 are graphically presented in Figure 1. According to the RSA% indicator, presented as TE, we obtained values of 7.24 ± 0.82 in the lumbar spine osteoporosis group; 6.79 ± 1.16 in the hip osteoporosis group; 5.46 ± 0.04 in the control group (Table2).

Table 1. Mean serum levels of RSA % , Mg, Cu, X mean ± SD

	Controls	Patients with osteoporosis with reduced hip bone density	Patients with osteoporosis with reduced bone density of the lumbar spine
Number of subjects examined	n = 14	n = 14	n = 37
BMD g/cm^2	1.13 ± 0.13	0.602 ± 0.49	0.729 ± 0.043
RSA%	55.67 ± 1.38	68.87 ± 13.26	73.30 ± 9.84
Age	62.29 ± 8.34	65.71 ± 6.43	62.78 ± 7.91
BMI, kg/m^2	28.86 ± 4.74	21.75 ± 3.33	24.44 ± 4.07
Mg, mmol/l	0.82 ± 0.07	0.96 ± 0.22	0.92 ± 0.19
Cu, µmol/l	19.74 ± 3.42	21.69 ± 5.21	21.11 ± 5.7

Table 2. t-test, P < 0.05 for statistical significance

Parameter	Patients with hip osteopenia vs controls	Patients with lumbar spine osteopenia versus controls	Patients with hip osteoporosis versus lumbar spine osteoporosis
BMD g/cm^2	P < 0.05	P < 0.05	P < 0.05
RSA %	P > 0.05	P < 0.05	P > 0.05
Възраст	P > 0.05	P > 0.05	P > 0.05
BMI, kg/m^2	P < 0.05	P < 0.05	P < 0.05
Mg, mmol/l	P < 0.05	P < 0.05	P > 0.05
Cu, µmol/l	P > 0.05	P > 0.05	P > 0.05

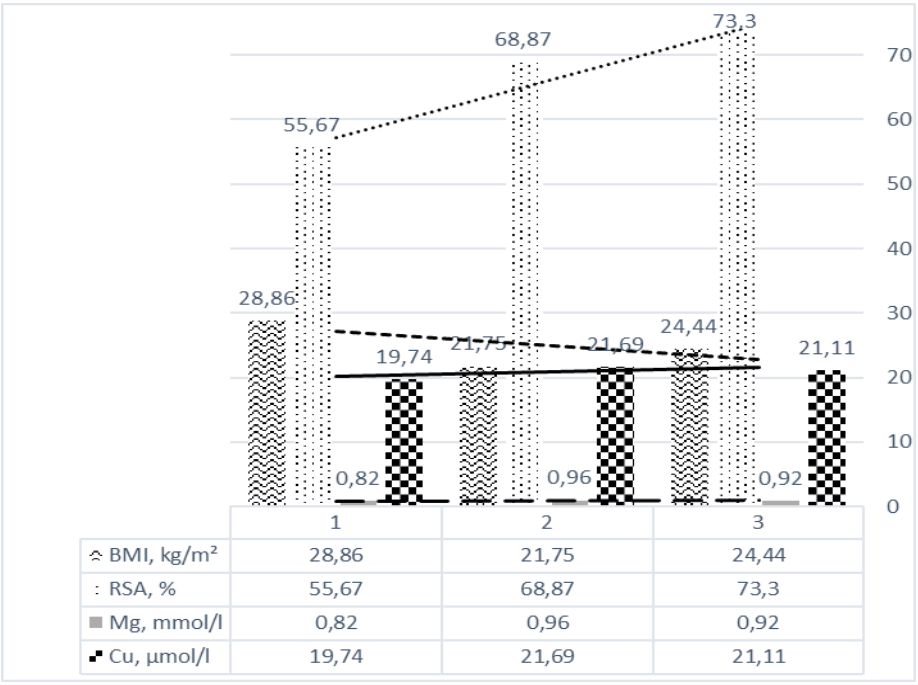

Figure 1. Mean values of BMI, RSA%, Mg, Cu, for the 3 groups (1 – control group, 2 – patients with osteoporosis of the hip, 3 – patients with osteoporosis of the lumbar vertebrae)

Discussion

The obtained results of the t-test according to age and BMD indicators confirm the correct selection of the studied participats. According to the BMI indicator, the groups have statistically significant differences and confirm what has been proven by previous researchers, that with a BMI > 27, the probability of having osteoporosis significantly decreases (Boyanov, 2006).

Serum copper and magnesium concentrations of all patients were elevated relative to the controls, but there was no statistical difference between groups with different disease localization. Patients with osteoporosis were found to have a higher antioxidant activity в сравнение с контролите. When monitoring the radical scavenging

activity, depending on the localization of the disease, we detected higher values in patients with osteoporosis of the lumbar spine.

Conclusions

Patients with osteoporosis have higher antioxidant activity in response to increased free-radical processes in the body

As bone density decreases, the level of serum RSA increases, as a result of increased ROS production. The higher level of radicals probably enhances the production of liver enzymes such as Cu, and Zn-SOD. On the other hand, the kinetics of radical reactions give us reason to consider that the overproduction of radicals damages the protein structure of oxidoreductases, especially in patients with slower biochemical mechanisms.

The serum concentration of copper is elevated in osteoporosis patients and leads to increased RSA

During the destruction of metalloproteins, redox-active metal ions are released. The higher level of radicals also damages cell membranes, making it easier for free copper to pass into the blood. The increased concentration of copper ions in the serum initiates secondary radical processes and further increases the patients' RSA.

The localization of osteoporosis determines the degree of imbalance of the redox state

The difference in BMD of the lumbar spine and the hip determines a different degree of imbalance of the redox state, RSA.

RSA% as a marker for localization of osteoporosis

To establish RSA% as a marker for localization of decreased bone density, it is necessary to: increase the number of participants; select participants with close values of BMI.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgments

This article was supported by the Research Fund of the Pleven Medical University under Grant № 14/2023 and was presented as an oral presentation at the International Conference on Medical, Health and Life Sciences (www.icmehels.net) held in Budapest/Hungary on July 06-09, 2023.

References

Asenova, Sv., & Andreeva-Gateva, P. (2020). Osteoporosis prevention by micronutrients - Current pathophysiological aspects. *General Medicine, 22*(2), 66-72.

Boyanov, M. (2006). *Klinichna rentgenova denzitometriya i kolichestven ultrazvuk na kostite*. Tshentralna meditshinska biblioteka. MU: Sofia.

Kwon, H. Y., Choi, S. Y., Won, M. H., Kang,T., & Kang, J.H. (2000) Oxidative modification and inactivation of Cu,Zn-superoxide dismutase by 2,2′-azobis(2-amidinopropane) dihydrochloride. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 1543*(1), 69-76.

Lazarova, D., Shibata, S., Ishii, I., Zlateva, G., Zhelev, Z., Aoki, I., Higashi, T., & Bakalova, R. Nitroxide-enhanced magnetic resonance imaging of kidney dysfunction in vivo based on redox-imbalance and oxidative stress. *Gen Physiol Biophys.*, *38*(3),191-204.

Mazzanti, L., Battino, M., Nanetti, L., Raffaelli, F., Alidori, A., Sforza, G., & Carle, F. (2015). Effect of 1-year dietary supplementation with vitaminized olive oil on markers of bone turnover and oxidative stress in healthy post- menopausal women. *Endocrine*, *50*(2), 326.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*, *26*(9-10), 1231.

Román, F., Urra, C., Porras, O., Pino, A. M., Rossen, C. J., & Rodríguez, J. P. (2017). Real-time H_2O_2 measurements in bone marrow mesenchymal stem cells (MSCs) show increased antioxidant capacity in cells from osteoporotic women. *J. Cell. Biochem.*, *118*(3), 585.

de Romaña, D.L., Olivares, M., Uauy, R., & Araya, M. (2011). Risks and benefits of copper in light of new insights of copper homeostasis. *J. Trace Elem. Med. Biol.*, *25*(3–13).

Rondanelli, M., Peroni, G., Fossari, F., Vecchio, V., Faliva, M. A., Naso, M., Perna, S., Di Paolo, E., Riva, A., Petrangolini, G., Nichetti, M., & Tartara, A. (2021). *Nutrients.*, *13*(3), 1012.

Sakac, V., & Sakac, M. (2000). Free oxygen radicals and kidney disease—part 1. Med Pregl., *53*(9-10), 463-474.

Croatian Schieber, M., & Chandel, N.S. (2014). ROS function in redox signaling and oxidative stress. *Curr Biol.*, *24*(10), 453-462.

Tomova, R., Asenova, S., Atanasova, B., Tzoneva, K., & Slavova. M. (2020). Flame atomic absorption determination of serum copper and zinc in disordered bone metabolism. *Bulgarian Chemical Communications*, *52*(1), 76-80.

Tomova, R., Asenova, S., Nestorova, R., Atanasova, B., Atanasova, L., Nikolova, M., Slavova, M. Multivariate statistical analysis for assessment of the relationships between bone density, biogenic elements content, and the level of oxidative stress in osteoporotic women. *Journal of IMAB*, *28*(4),4742-4748.

Valko, M., Jomova, K., Rhodes C.J., Kuča K, & Musílek, K. (2016). Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol.*, *90*(1), 1-37.

Valko, M., Morris, H., & Cronin, M. T.D. (2005). Metals, toxicity and oxidative stress. *Curr Med Chem.*, *12*(10),1161-1208.

Wu, X., Li, J., Zhang, H., Wang, H., Yin, G., & Miao, D. (2017). Pyrroloquinoline quinone prevents testosterone deficiency-induced osteoporosis by stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption. *Am. J. Transl. Res.*, 9(3), 1230.

Author Information

Radka Tomova
Medical University of Pleven
Pleven, Bulgaria
Contact e-mail: rtomova@mail.bg

Svetla Asenova
Medical University of Pleven
Pleven,Bulgaria

Lilia Atanasova
Medical University of Sofia
Sofia, Bulgaria

Rodina Nestrova
Rheumatology Centre St. Irina
Sofia,Bulgaria

Bisera Atanasova
Medical University of Sofia
Sofia, Bulgaria

Tzvetanka Petranova
Medical University of Sofia
Sofia, Bulgaria

Mariana Nikolova
Middlesex University
Bulgaria

Miglena Slavova
Institute of Electrochemistry and Energy Systems
Bulgaria

Radka Tafrajijska-Hadjiolova
Medical University of Sofia
Bulgaria

To cite this article:

Tomova, R., Asenova, S.., Atanasova, L., Nestrova, R., Atanasova, B., Petranova, T., Nikolova, M., Slavova, M., & Tafrajijska-Hadjiolova, R. (2023). Redox state and biogenic elements in osteopororis with different localization. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 10,* 49-54.