THE TRINITY OF ESSENTIAL TRACE ELEMENTS (TES) IMBALANCE, OXIDATIVE STRESS (OS) AND INFLAMMATORY STATE IN CHRONIC KIDNEY DISEASE AND HEMODIALYSIS (HD) PATIENTS: VICIOUS TRIAD FOR DISEASE PROGRESSION AND OUTCOMES

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Abstract: Chronic kidney disease (CKD) is a socially significant disease. Worldwide, CKD prevalence is increasing, with it expected to become the fifth most common chronic disease by 2040. In end-stage renal disease (ESRD), the kidneys are unable to cleanse the body of metabolic waste products. Global Burden of Disease (GBD) studies show that despite the decline in the ESRD mortality, CKD is emerging as a leading cause of death on the worldwide scale. The global epidemiology of ESRD reflects the unique genetic, environmental, lifestyle and socio-demographic characteristics of each nation. Dialysis treatment is a long-term replacement therapy allowing the body to continue functioning when the vital excretory system stops working properly. Renal replacement therapy (RRT) reduces uremic intoxication, maintaining the body's internal environment in a state, closest to the physiological. The predominant type of RRT in most countries is dialysis, with hemodialysis (HD) being the most common one. Maintenance hemodialysis (MHD) is one of the greatest achievements of medicine in the second half of the last century, thanks to which thousands of people succeed to return to an almost normal lifestyle with satisfying social integration and participation. Despite offering years of life, it is reported that HD is a major cause of essential elements imbalances. Minerals, like all matter, are made out of chemical elements, including small-amount elements and elements, present in the human body in diminutive quantities. Knowledge about micronutrient status in CKD is still limited. In recent years, data on the pleiotropic effects of TE in maintaining multisystem physiological balance are increasing, accentuating on their role as an epigenetic factor, as well as on the key importance of TEs for environmental and occupational health. Microelements have an essential role for the optimal biological, chemical, energetic and molecular cellular functioning. They are involved in vital biochemical reactions by acting as cofactors for many enzymes. Elemental balance is of key importance for the physiological immune response, accordingly, the state of deficiency can be crucial for the development and progression of various diseases, among which: CKD. It is found that in dialysis and pre-dialysis patients, zinc (Zn) and selenium (Se) are the two major elements that are significantly depleted as compared to normal subjects. Over the past few decades, a large number of clinical, experimental, and theoretical data state signs of oxidative stress (OS) in ESRD patients, which is considered a biochemical hallmark of CKD, affecting the progression and deterioration of renal function, as well as the occurrence of systemic comorbidities such as cardiovascular disease (CVD). Dietary restrictions, comorbidity, medical therapy, MHD, play a key role in the inadequate intake, unpredictable absorption, impaired metabolism, and increased elemental losses among these patients. Zn, Cu and Se, being involved in the molecular structure of some of the most powerful antioxidant systems, are of particular interest, as they can only be supplemented exogenously, thus contributing to sustainable quality of life in the presence of CKD. Providing personalized medical care would support the diagnosis, as well as modern therapy aspects of a number of diseases associated with TEs imbalance, especially CKD. Conclusive research may provide more successful and comprehensive approach to CKD management, hence the aim of this review to contribute for the establishment of proper supplementation strategies and reliable laboratory diagnosis of elemental imbalance in CKD and HD patients.
Keywords: CKD, trace elements, oxidative stress, dialysis, zinc, copper, selenium

1. INTRODUCTION

Chronic kidney disease (CKD) is an important public health concern. The prevalence of CKD is about 10% to 14% in the general population, but the true incidence is difficult to be determined because of the sometimes asymptomatic early stages of the disease. Worldwide, about 2.6 million people are with CKD. Among the elderly

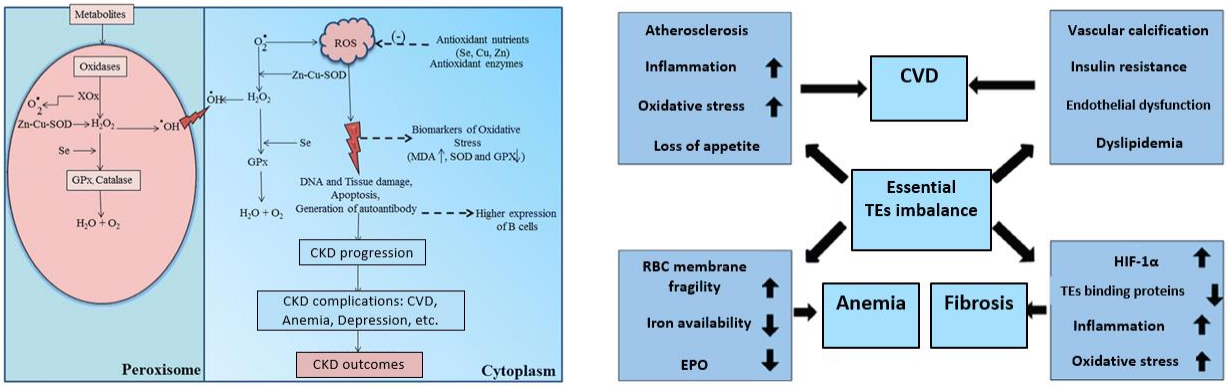
population in Bulgaria, about 3 000 patients annually receive RRT, with about 89% undergoing HD and 11% peritoneal dialysis. In the past decade, the number of dialysis patients is increasing 3 to 5% per year. Current international guidelines define CKD as decreased kidney function shown by a glomerular filtration rate (GFR) less than 60 mL/min/1.73 m2, and/or markers of kidney damage of at least 3 months duration, regardless of the underlying cause. It is classified based on 3 categories: cause of CKD, GFR (G1-G5) and level of albuminuria (A1-A3). Three main reasons are highlighted regarding its manifestation: diabetes mellitus type 2 (30% to 50%), arterial hypertension (27.2%) and on third place are all other kidney diseases, the progression of which leads to end-stage renal disease (ESRD). When kidney's normal excretory function becomes insufficient as a result of CKD, HD is one of the interventional treatment methods enabling the preservation of vital functions. Based on a concentration gradient across a semi-permeable membrane between patient's plasma and dialysate solution, HD removes uremic toxins. However, despite offering years of life, it is reported that HD is a major cause of essential elements imbalance 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0. During the procedure, there is a great exchange of substances between blood plasma water space and dialysate, with water-soluble small-size solutes passing from blood to dialysate, while others move from dialysate to blood 0, 0. Depletion of essential substances can occur either if their level in the dialysate solution is lower of that in the blood, or if they are not present at all in the dialysate. As for essential trace elements (TEs), imbalance may be due to low intake as a result of dietary restrictions, anorexia, or as a consequence of the disease process itself. Studies on the protein binding of TE and their behavior during dialysis are scarce 0, but low binding rates are reported for Se and Zn (25 % and 12–17%), making them more susceptible to loss during a dialysis procedure 0. Disease progression and clinically significant implications of various body's systems (incl. renal function impairment, increased risk of cardiovascular disease, anemia, cancer, immune deficiency), following TEs imbalance and disproportionate accumulation, are known to contribute for poor outcomes or to have fatal consequences in the general population. While zinc deficiency clinically manifests with anemia, delayed wound healing, and reproductive system abnormalities, selenium deficiency is associated with cardio vascular diseases (CVDs), compromised function of the immune system, sleep disorders. There is increasing emphasis on the importance of complex elemental imbalance. It is known that a decrease in Zn concentrations is accompanied by an increase in blood Cu, from where the potential diagnostic value of the Cu/Zn ratio. The copper/zinc ratio as a biomarker is essential in various disease states, especially CKD and its progression. Zn, Cu and Se, being involved in the molecular structure of some of the most powerful antioxidant systems, are of critical importance for the immune defense and the mitigation of OS. They mediate vital biochemical reactions, acting as cofactors of many enzymes, among which: catalase (CAT), glutathione peroxidase (GPX) and Cu, Zn superoxide dismutase (SOD), which eliminate reactive oxygen species (ROS) from the cell, prevent tissue and DNA damage, cell apoptosis, and synthesis of autoantibodies. Until recently, un increasing number of studies, focused on TEs imbalance (incl. Zn, Cu and Se), have demonstrated that they affect the degree of OS, which has been shown to be closely related to the inflammatory status in CKD and MHD patients 0, 0, 0. Dialysis patients are prone to deficiency of essential TEs, depending on dietary intake, residual kidney function, uremia-induced hyper catabolism, persistent inflammation, removal during HD, TEs content of the dialysate and the water used for dialysis. Considering all the factors affecting TE status in CKD patients, we can conclude that this group is exceptionally vulnerable to essential elements imbalance. Still, very little is known about TEs concentration, its metabolism and pathophysiology in normal healthy individuals and uremic patients. It is delineated that the loss of trace elements balance in ESKD patients significantly contributes to increased morbidity and mortality. However, further research on the role of TEs as a factor for disease incidence, progression and complications, is needed. Considering eventual elemental imbalance during all stages of CKD, would support optimal immune function, reducing the impact of OS and improving the life quality of patients with CKD. The aim of this review article is to summarize the role of Zn, Cu and Se status in CKD and ESRD, raising physician's awareness of the essential elements imbalance among this group of patients. This would arouse more comprehensive approach of TEs requirements and supplementation, reducing the impact of OS and improving the overall life quality of CKD and dialysis patients.

2. TEs, OS AND IMMUNE SYSTEM: A VICIOUS TRIAD IN CKD AND HD PATIENTS

Depending on their total body content, bioelements are classified into three groups: macroelements, microelements, and ultramicroelements. Macroelements are five basic structural elements that construct carbohydrates, proteins and lipids in all tissues: nitrogen (N), carbon (C), hydrogen (H), sulfur (S) and oxygen (O), as well as the electrolytes potassium (K), sodium (Na), chlorine (Cl), calcium (Ca), magnesium (Mg) and phosphorus (P). The daily intake of these elements is over 100 mg. The remaining elements, whose concentration in biological fluids is of the order of 10 μg/L to 10^4 μg/L, are designated as microelements, and those with a concentration <10 μg/L - as ultramicroelements. Essential elements, vital to the human body, include copper (Cu), zinc (Zn), selenium (Se), iron (Fe), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), molybdenum (Mo), etc. The main criteria for

determining an element as essential for human health, is the appearance of metabolic, functional or structural changes as a result of a deficiency or reduced intake of the corresponding element. In the same time, all these abnormalities are reversible upon restoration of the nutrient intake. CKD and HD patients are with impaired protein homeostasis and metabolism, acid-base disorders, hormonal dysfunctions. With the progression of the disease, nitrogen-containing products accumulate, which leads to nutritional disorders, decrease in appetite and anorexia. In addition, intestinal absorption is also compromised as uremia causes microbiota disturbance and damage to the intestinal epithelium 0. Acting in a synergetic way, all these factors make the CKD patient's nutritional status irregular, often resulting in a loss of protein energy and TEs. According to literature data, the essential elements with disturbed blood concentration levels that most often accompany CKD and HD patients are Zn, Cu, and Se 0. Zn is important for maintaining the activity of over 300 enzymes involved in the controlling mechanisms of cell replication, membrane integrity, bone formation, growth, sexual maturation and fetal development. Cu is an essential cofactor of a group of metalloenzymes, and its optimal homeostasis is of particular importance for the proper immune system functioning, erythropoiesis, skeletal development, melanogenesis and pigmentation. Se is a component of a group of 25 selenoproteins that contain selenocysteine in their molecular structure, including glutathione peroxidase and thioredoxin reductases, which are major antioxidant enzymes. The classic role of mitochondria is the production of ATP (adenosine triphosphate) through oxidative phosphorylation-process, constantly generating superoxide anions (O^{2-}) in healthy physiologic conditions. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are the three major antioxidant systems, imperative for the O^{2-} scavenging and the whole ROS elimination strategy. Enzymatic and non-enzymatic antioxidants, composing the entire body's defense system, act cooperatively to resist the multiplex free radicals adverse effects. Cu, Zn, and Se are essential as activators of many enzymes, especially the above mentioned, included in the first line of defense: SOD, CAT and GPX. A lot has been published concerning antioxidants and their significance in preventing OS, notwithstanding with scantiness of awareness on the fundamental role of TEs, indispensable in the entire defense strategy of antioxidants. The present review endeavor to articulate important information on the significance of Cu, Zn, Se and their involvement in the degradation of ROS in the context of CKD and HD patients.The role of these TEs in maintaining the balance between oxidant and antioxidant processes in the cell is indirect but of key importance. OS occurs when this balance is disturbed in favor of an increasing concentration of reactive oxygen radicals (ROS). Approximately 4–5% of the oxygen used in oxidative metabolic reactions is converted to ROS, primarily superoxide anions. SOD catalyzes the conversion of superoxide anions to hydrogen peroxide (H_2O_2), but in the presence of partially reduced metal ions (Fe^{2+}), H_2O_2 reacts with them to form hydroxyl radicals. They, in turn, are highly reactive and can interact with nucleic acids, lipids and proteins. The involvement of Cu, Zn, and Se in the formation and degradation of free radicals in the cell and the mechanism by which their deficiency induces DNA and tissue damage, cell apoptosis, and autoantibody synthesis are illustrated and detailed below in (**Fig.1)**.

Fig.1 TEs involvement in ROS and OS generation/degradation. CKD complications due to OS and TEs imbalance.



The entire figure represents the essential role of TEs for the maintenance of redox homeostasis, which once disturbed in favor of oxidants (O^{2-}, generated by mitochondrial enzyme XOX), increases OS, consecutively leading to CKD progression and further complications. On the figure's left side, mitochondrial production of H_2O_2 is shown, along with its fate: decomposition by CAT and GPX or conversion to (OH·). Generated O^{2-} are scavenged by SOD. Impaired overall antioxidant capacity results in lipid peroxidation and O^{2-} and OH· penetration of the cell membrane, which eventually cause DNA and tissue damage, increased autoantibody synthesis, disease progression, complications and unfavorable outcomes. The role of essential TEs imbalance and their vicious interaction with OS

and immune status in CKD is emphasized on the right side of the figure. TEs, trace elements; CKD, chronic kidney disease; OS, oxidative stress; O^{2-}, superoxide anions; XOX, xanthine oxidase; H_2O_2, hydrogen peroxide; CAT, catalase; GPX, glutathione peroxidase; SOD, superoxide dismutase; (OH·), hydroxyl radicals; DNA, deoxyribonucleic acid; ↑, increase; ↓, suppression; EPO, erythropoietin; HIF-1α, Hypoxia-inducible factors-1α.

In healthy individuals ROS and RNS (reactive nitrogen species) production is apparent to a slight extent, as oxidants are involved in the activation process of WBC (white blood cells: lymphocytes, monocytes, T-cells), signaling pathways, cell differentiation and proliferation, as well as in the complex functionality of the adaptive system 0, 0. Therefore, the above mentioned antioxidant mechanisms emerge of paramount importance to balance body's physiological oxidants production, thus maintaining the redox homeostasis. Throughout this entire precise regulation, an adequate intake of TEs, as recommended for healthy individuals, is fundamental, yet still omitted or often underestimated, especially in CKD-disease, associated with pathophysiological changes in the oxidants-reductants balance. Studies had proven that antioxidant defense mechanisms are compromised among CKD patients, showing a decrease of antioxidants, along with stimulated generation of ROS an RNS by WBC and cell metabolism under anaerobic conditions. Reduced plasma levels of CAT, GPX, intracellular glutathione, and thiol have been reported. Regarding SOD, decreased expression of the SOD gene have been observed. Moreover, genetic polymorphism in another antioxidant enzyme, glutathione-S transferase, is also shown to increase OS in CKD and HD patients 0. Impaired overall antioxidant capacity in CKD and HD patients may be assumed as a consequence of increased loss of TEs, essential for its integrity (e.g., due to anorexia, impaired absorption and metabolism, increased urinary excretion, medical procedures, incl. RRT, the use of medications), or antioxidants redirection and involvement in distant metabolic pathways and immunologic reactions. Progressively, generated OS cause mitochondrial structural changes-foundation for a renewed and self-sustaining oxidants production, eventually resulting in organ dysfunctions, aggravation of overall outcomes and CKD complications.

3. CKD COMPLICATIONS DUE TO OS AND TEs IMBALANCE

With renal function deterioration and GFR progressive reduction, patients with CKD may develop a number of complications, the manifestation of which is associated with OS as an important risk factor. The increased risk of CVD stand out, together with a rise of its morbidity and mortality. Risk factors for CVD events in CKD patients are considered traditional and non-traditional. Traditional risk factors include diabetes mellitus (DM), aging, hypertension, hyperlipidemia, smoking, etc. Non-traditional risk factors include OS, inflammation, etc. The increased risk of CVD is directly proportional to the progression of CKD, with stage five-ESRD patients on MHD-exhibiting the highest risk. This specific population show two interrelated conditions, inflammation and OS, directly associated with each other. Increased serum proinflammatory marker CRP (c-reactive protein) is related to an increased risk of cardiovascular mortality in patients undergoing MHD. Together with interleukin-6 (IL-6), it is reported in a positive correlation with malondialdehyde (MDA) in CKD patients, along with a negative association with endogenous antioxidants enzymes, including SOD and GPX. The association of essential TEs imbalance with CVD, anemia, and fibrosis in CKD is illustrated in (**Fig.1)**.

4. CONCLUSIONS

Malnutrition, insufficient micronutrient intake, and increased losses of vitamins and TEs in the dialysis process jeopardize antioxidant defense mechanisms in CKD. Interventions that aim to alleviate OS and chronic inflammation in CKD patients are considered potential managing options to improve treatment outcomes in this population. Antioxidants and minerals, especially Zn and Se, have emerged as plausible therapeutic agents due to their activities that target the highly oxidative milieu in CKD patients and improve the pro-inflammatory biomarkers. In the light of the present literature review, further studies are needed. This would raise awareness and facilitate access to highly sensitive instrumental techniques for multi-elemental analysis, allowing the establishment of new aspects in the pathogenetic, diagnostic and therapeutic approach of TEs imbalance.

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REFERENCES

Almeida, A., Gajewska, K., Duro, M., Costa, F., & Pinto, E. (2020). Trace element imbalances in patients undergoing chronic hemodialysis therapy–report of an observational study in a cohort of Portuguese patients. *Journal of Trace Elements in Medicine and Biology*, *62*, 126580.

Azevedo, R., Gennaro, D., Duro, M., Pinto, E., & Almeida, A. (2023). Further Evidence on Trace Element Imbalances in Haemodialysis Patients—Paired Analysis of Blood and Serum Samples. *Nutrients*, *15*(8), 1912.

Berger, M. M., Broman, M., Forni, L., Ostermann, M., De Waele, E., & Wischmeyer, P. E. (2021). Nutrients and micronutrients at risk during renal replacement therapy: a scoping review. *Current opinion in critical care*, *27*(4), 367.

Chen, C. H., Huang, S. C., Huang, S. W., Tsai, S. F., & Huang, Y. C. (2023). Trace Elements Status and Their Associations With Related Antioxidant Enzyme Activities in Patients Receiving Peritoneal Dialysis and Hemodialysis. Journal of Renal Nutrition.

Dresen, E., Pimiento, J. M., Patel, J. J., Heyland, D. K., Rice, T. W., & Stoppe, C. (2023). Overview of oxidative stress and the role of micronutrients in critical illness. *Journal of Parenteral and Enteral Nutrition*, *47*, S38-S49.

Dsouza, A. P., Reddy, R., Yadav, A., & Mala, M. (2019). Effect of Hemodialysis on Trace Elements in Renal Failure Patients. *Indian Journal of Medical Biochemistry*, *23*(2), 234.

Filler, G., & Felder, S. (2014). Trace elements in dialysis. *Pediatric Nephrology*, *29*, 1329-1335.

Fleming, G. M. (2011). Renal replacement therapy review: past, present and future. *Organogenesis*, *7*(1), 2-12.

Fukasawa, H., Furuya, R., Kaneko, M., Nakagami, D., Ishino, Y., Kitamoto, S., ... & Yasuda, H. (2023). Clinical significance of trace element zinc in patients with chronic kidney disease. *Journal of Clinical Medicine*, *12*(4), 1667.

Harun, H., & Pradana, G. (2023). The Role of Nutritional Therapy in Inhibiting the Progression of Chronic Kidney Disease: A Narrative Literature Review. *Bioscientia Medicina: Journal of Biomedicine and Translational Research*, *7*(3), 3178-3184.

Hasanato, R. M. (2014). Assessment of trace elements in sera of patients undergoing renal dialysis. *Saudi Med. J*, *35*(4), 365-70.

Hossain, M. S., Amin, M. N., Das, A., Khan, A. J. H., Sohel, M., Ahmed, J., ... & Islam, M. S. (2021). Increased lipid peroxidation, depleted non-enzymatic antioxidant, and variability in trace elements concentration in serum are correlated with Bangladeshi end-stage renal disease population. *Health Science Reports*, *4*(3), e348.

Karkar, A. (2013). Advances in hemodialysis techniques. In *Hemodialysis*. IntechOpen.

McClave, S. A., Wischmeyer, P. E., Miller, K. R., & van Zanten, A. R. (2019). Mitochondrial dysfunction in critical illness: implications for nutritional therapy. *Current Nutrition Reports*, *8*, 363-373.

Prodanchuk, M., Makarov, O., Pisarev, E., Sheiman, B., & Kulyzkiy, M. (2013). Disturbances of trace element metabolism in ESRD patients receiving hemodialysis and hemodiafiltration. *Central European journal of urology*, *66*(4), 472.

Radic Savic, Z., Coric, V., Vidovic, S., Vidovic, V., Becarevic, J., Milovac, I., ... & Simic, T. (2023). GPX3 rs8177412 Polymorphism Modifies Risk of Upper Urothelial Tumors in Patients with Balkan Endemic Nephropathy. *Medicina*, *59*(8), 1421.

Shanmugam, L., Green, S. R., Radhakrishnan, H., Kadavanu, T. M., Ramachandrappa, A., Tiwari, S. R., ... & Govindasamy, E. (2016). Trace elements in chronic haemodialysis patients and healthy individuals-A comparative study. *Journal of Clinical and Diagnostic Research: JCDR*, *10*(10), OC14.

Supriyadi, R., Koswara, M. I. A., Soelaeman, M. A., & Huang, I. (2023). The effect of antioxidants supplementation on oxidative stress and proinflammatory biomarkers in patients with chronic kidney disease: a systematic review and meta-analysis. *European Review for Medical & Pharmacological Sciences*, *27*(4).

Stojsavljević, A., Ristić-Medić, D., Krstić, Đ., Rovčanin, B., Radjen, S., Terzić, B., & Manojlović, D. (2022). Circulatory imbalance of essential and toxic trace elements in pre-dialysis and hemodialysis patients. *Biological trace element research*, 1-9.

Tonelli, M., Wiebe, N., Bello, A., Field, C. J., Gill, J. S., Hemmelgarn, B. R., ... & Alberta Kidney Disease Network. (2017). Concentrations of trace elements in hemodialysis patients: a prospective cohort study. *American Journal of Kidney Diseases*, *70*(5), 696-704.

Xu, Y., Li, A., Li, X., Deng, X., & Gao, X. J. (2023). Zinc deficiency induces inflammation and apoptosis via oxidative stress in the kidneys of mice. *Biological Trace Element Research*, *201*(2), 739-750.