

SERUM TRACE ELEMENTS IN PATIENTS WITH CARDIOVASCULAR DISEASE

Afrim TABAKU¹, Engjëllushe HASANI², Entela BUSHI³

¹Pharmacotherapeutic Research Center; Faculty of Medical Sciences; Aldent University; Tirana, Albania

²Health Center Nr. 3, Tirana, Albania

³Public Health Institute Tirana-ALBANIA

(Corresponding author: afrim.tabaku@ual.edu.al)

Abstract

INTRODUCTION: Many trace elements play important roles in activating or inhibiting enzymatic reactions, by competing with other elements and metal proteins for binding sites, by affecting the permeability of cell membranes and by other mechanisms. They play important roles in the oxidant/antioxidant balance. As such, trace elements are thought to be involved directly or indirectly in the pathogenesis of several diseases.

Free radicals have harmful effects on cells and tissues and are thought to be responsible for the pathogenesis of many diseases. Trace elements are required for the antioxidant enzymes and hence the optimal function of the immune system. Changes in the levels of these elements may lead to a reduction in antioxidant activities in various diseases.

The aim of this survey is to put in evidence possible alterations of copper, zinc and selenium status in patients suffering by oxidative stress caused by cardiovascular disease (CVD).

MATERIAL AND METHODS: We investigated serum levels of copper, zinc, and selenium in 83 patients hospitalized by acute myocardial infarction in some hospitals in Tirana and in 70 healthy persons. Serum copper and zinc measurements were carried out using flame atomic absorption spectrometry Shimadzu 7700 (AAS) and serum selenium measurements were carried out using atomic absorption spectrometry hydride method. Statistical processing of the data was carried out using Statistical Package for Social Science (SPSS 20).

RESULTS AND DISCUSSION: The results of this study have shown that levels of copper have increased in patients compared to control group, while the levels of zinc, and selenium decreased in patients compared to control group. Significantly low serum zinc ($p < 0.001$) and serum selenium ($p < 0.001$) were found in patients suffering by oxidative stress caused by CVDs compared to the group of healthy persons, whereas significant difference between the groups included in our survey was also found for serum copper ($p < 0.01$).

CONCLUSION: The results of this study indicate that there are alterations in serum concentrations of trace elements in cardiovascular patients, suggesting that they may play a role in the pathophysiology of these diseases by virtue of their role in oxidative stress.

KEYWORDS: CVD, serum zinc, serum selenium, serum copper, oxidative stress.

Introduction

Heart disease is one of the major health problems of developing countries of the world. Oxidative stress plays an important role in the progression of adverse complications in CVD, and many trace elements are involved in the oxidant-antioxidant balance. Essential trace elements copper, zinc and selenium are important parts of antioxidant enzymes as superoxide dismutase, glutathione peroxidase as well as of transport protein with antioxidant properties-ceruloplasmin. Mentioned trace elements may affect antioxidant defence system. Abnormalities associated with trace elements have not received much attention from clinicians in the past; however, in the past few years there has been a veritable explosion of knowledge about trace elements which are associated with abnormalities in experimental animals as well as in humans. The information explosion is rapidly reaching the stage where clinicians will be called upon more frequently to diagnose and treat trace element-related diseases (Linder and Hazeghazman, 1996, Kralik et al, 1996, Cordova and Alvarerez-Mon 1995).

Normal cardiovascular function is affected profoundly by a large number of processes at the molecular level. Many other etiological factors play a role. In the human body, trace elements function in a similar way; most of them are found at the active sites of enzymes or of physiologically active substances of the body.

Dietary deficiency causes a variety of clinical signs and symptoms through the decreased activity of these active substances. Slight or severe trace elements imbalances are considered risk factors for coronary heart disease (Metz 1982, Wada and Yanagisawa 1996).

Many elements exert a very strong influence on CVD risk factors such as disorders of blood lipids, blood pressure, coagulation, glucose intolerance and circulating insulin.

Detection and correction of trace elements imbalance in populations reduce the incidence of atherosclerotic heart disease by diminishing individual risk factors. Recent developments suggest that marginal deficiencies of microelements are common in human nutrition.

Copper, zinc and selenium are essential elements that have an important role in protection against oxidative stress, which has been implicated in the pathogenesis of over 100 human diseases (Halliwell and Cross 1994, Telisman 1995, Cerrutti, Ghosh, Oya, Amstad 1994). Stress can affect many vital processes and increase individual susceptibility to various diseases (Chrouspos and Gold 1992, Ader et al, 1995)

The aim of this survey is to put in evidence possible alterations of copper, zinc and selenium status in patients suffering by oxidative stress caused by CVD

Materials And Methods

We estimated serum copper, zinc and selenium levels of 83 patients hospitalized by acute myocardial infarction in some hospitals in Tirana, as well as 70 healthy persons as the control group. Blood samples were collected by vein puncture in plastic tubes. The samples were then centrifuged (2000 g at 4^o C for 10 min) immediately. The sera were stored in - 20^o C until subsequent analysis.

Serum copper and zinc measurements were carried out using Shimadzu AA 7000 flame atomic absorption spectrometry and serum selenium measurements were carried out using atomic absorption spectrometry hydride method. Statistical processing of the data was carried out using Statistical Package for Social Science (SPSS 20).

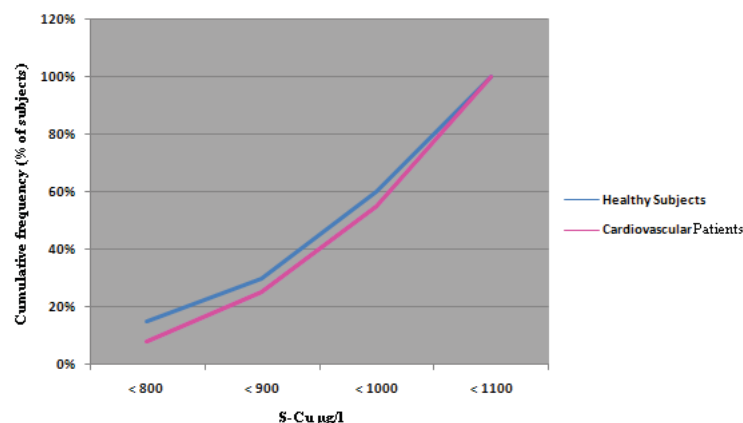
Results And Discussion

Data on levels of copper, zinc and selenium in sera are summarized in tables 1-3 and data on the

cumulative frequency distribution are presented by the respective graphs.

Table 1. Levels of Cu in sera of cardiovascular patients and of control group ($\mu\text{g/L}$)

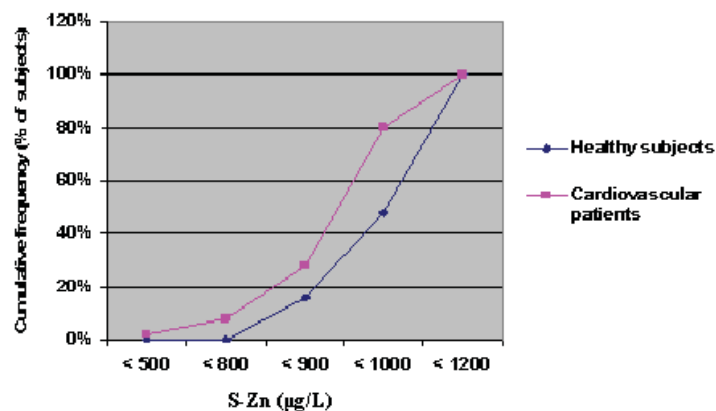
	Patients group	Control groups	Significance
Range	772 - 1105	698 - 1062	P < 0.01
Mean \pm SD	981.85 \pm 12.20	815.55 \pm 18.11	
Median	964.18	769.45	
CI 95%	925.82 - 985.21	788.41 - 824.57	
Number of samples	83	70	



Graphic 1. The cumulative frequency distribution of the copper serum (S-Cu) in healthy subjects and cardiovascular patients.

Table 2. Levels of Zn in sera of cardiovascular patients and group of control ($\mu\text{g/L}$)

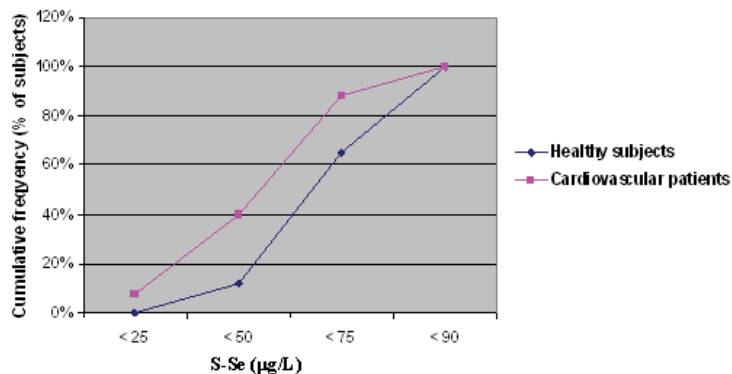
	Patients group	Control groups	Significance
Range	428-980	825-1170	P < 0.001
Mean \pm SD	790 \pm 32.86	995.04 \pm 18.85	
Median	786.34	1005.00	
CI 95%	723.23 - 856.46	956.14 - 1033.94	
Number of samples	83	70	



Graphic 2. The cumulative frequency distribution of serum zinc (S-Zn) in healthy subjects and cardiovascular patients.

Table 3. Levels of Se in sera of cardiovascular patients and group of control ($\mu\text{g/L}$)

	Patients group	Control groups	Significance
Range	14.20-80.60	41-88	P < 0.001
Mean \pm SD	46.98 \pm 3.44	65.84 \pm 2.063	
Median	45.90	65.60	
CI 95%	41.84 - 52.12	61.60 - 70.12	
Number of samples	83	70	



Graphic 3. The cumulative frequency distribution of the serum selenium (S-Se) in healthy subjects and cardiovascular patients.

As it is shown by tables and graphs, there is a difference on the concentration of microelements under survey between patients suffering by CVD and the control group. Obtained results shown a decrease of levels of serum zinc and serum selenium in patients compared to healthy control group, as well as an increase of levels of serum copper in patients versus healthy control group. Similar results have been obtained by other authors in their studies (Yahya et al, 2014).

The association between blood Zn and Cu concentration and prevalence of CVD has been described controversially (Cikim et al, 2003, Sinning et al, 2010). Previous reports demonstrating an association between low Zn and Cu concentrations and incidence of cardiovascular events in epidemiological studies but other recent studies revealed controversial data; for example in several prospective (Venardos et al, 2007, Venardos et al 2004, Rayman 2000) and retrospective (Blankenberg 2003) studies low serum Zn, Cu and selenium levels were associated with increased risk of coronary heart disease, however, in other studies this association could not be confirmed.(Navarro-Alarcon et al, 2000, Flores-Mateo et al, 2006, Bleys et al, 2006).

The controversies and complexities surrounding the oxidant/antioxidant role of copper zinc and selenium will be emphasised as there is now substantial evidence that some, but not all, of the potential role of changing of levels of these trace elements in cardiovascular mechanisms are related to oxidant stress and compromised antioxidant defence. Finally, the relationship between copper, zinc and selenium status and major biological mechanisms associated with CVD, especially atherosclerosis, arterial compliance and thrombosis will be evaluated using data from experiments in man and animals.

Recent research has shown that free radicals, particularly, reactive oxygen species (ROS) play an important role in the pathogenesis of oxidative myocardial damage with consequential cardiac malfunction (Bandyopadhyay et al 2004) Oxidative stress describes the condition where an excessive

production of ROS overwhelms endogenous antioxidant defence mechanisms. The resultant elevation in ROS levels has a detrimental effect on cellular function, a consequence of ROS-induced damage to lipid membranes, enzymes and nucleic acids. Generation of ROS has been involved in various cardiovascular disorders, including ischaemia/reperfusion (I/R), atherosclerosis and cardiotoxicity induced by drugs (Scolletta et al, 2007) These ROS caused an injury to vascular cells and cardiac myocytes directly, and can initiate a series of local chemical reactions that result in an amplification of the initial ROS-mediated cardiomyocyte dysfunction (Blankenberg et al 2003).

In-vivo antioxidant nutrients which include vitamin C, trace elements such as Se, Zn and Cu play a crucial role in defending against oxidant damage (Thangadurai et al, 2012). The results of published papers showed that the levels of trace elements (Zn, Se) of patients significantly decreased compared with control groups. The lowering in these values reflected the principal function of these elements as antioxidants (as free ion elements or bounding with enzyme) in biological systems (Paola et al, 2013). The decrease in zinc level and its concomitant effect on copper level may affect the activity of some antioxidant enzymes that use these elements as cofactor within its structure. Hence those enzymes lose some of their activity and ability to remove free radicals (Beck et al, 1997).

Human body uses selenium to produce glutathione peroxidase, which works with vitamin E to protect cell membranes from damage caused by dangerous, naturally occurring substances known as free radicals produced by oxidative metabolism, Selenium is taking center stage as a potential anticancer agent by promoting formation of white blood cells which destroy the cancer cells and are an essential component of more than ten selenoproteins with multiple biochemical functions. Moreover, it boosts the immune system by increasing the activity and number of white blood cells and prevents premature aging, degenerative diseases, CVDs, inflammatory

diseases, stroke, cataracts, and rheumatoid arthritis. It is also necessary for normal thyroid functions and protection of heavy metal toxicity. Deficiency of the element can cause Keshan disease, characterized by an enlarged heart and poor heart function (Čuparigova et al, 2011). Highly significant increase in copper level was observed in the sera of patients compared with control group. Among the cationic ligands, copper deserves particular consideration because it acts as a transition metal, and it is very potent to generate ROS after a reaction with oxygen. Free Cu (II) ion can interact with hydrogen peroxide (H_2O_2) leading to the formation of the deleterious hydroxyl radical via the Fenton reaction. Bound to proteins, copper is generally less susceptible to participate in the Fenton reaction (Marjolaine et al, 2008). In the reported literature the ranges of so-called "normal value" of these microelements are relatively large (Cornelis, Sabbioni, Van den Venne 1994, Alfthan and Neve 1996, Minoia et al. 1990). The concentration of microelements in sera of general population depended on the exposure to other metals and their interaction, as well as on different dietary habits, and the content of microelements in food (Telisman 1995, 1997, Golubkina and Alfthan, 1999).

We have not reported or published data about the levels of microelements in sera of population in our country. The scarce data that we have show that the concentration of these microelements is in general slightly lower for S-Zn and S-Cu and lower for S-Se compared with those reported in literature of other countries. The lower than normal value content of selenium in the serum of healthy persons perhaps depends on low concentration of selenium in soil or low bioavailability of the selenium for plants, and also on eating habits.

A study from Germany reported mean serum values of Cu (mg/l), Zn (mg/l) and Se ($\mu\text{g/l}$) in adults to be 1.048, 1.079 and 63.2, respectively (Rukgauer et al, 1997). Studies of Se levels in a healthy Spanish population showed a mean value of 80.7 $\mu\text{g/l}$ (Torra et al, 1997) which is very high compared to that of our survey. On the other hand, higher mean serum Se values (122 $\mu\text{g/l}$) were reported in a population from Singapore (Huges et al, 1998)

Our results showed that the levels of S-Zn and S-Se in cardiovascular patients were significantly lower than levels of the control group, whereas significant difference was found and for S-Cu, but in this case S-Cu levels in patients were significantly higher than levels of control group. These significant differences may be due to the consequence of increased oxidative stress caused by the disease.

This survey has several strengths; it is the only published study of the trace elements in patients with CVD in Albania. Other strength is the significant difference levels of trace elements between patients with CVD versus control healthy group and a well-selected control group. We would like to mention the weakness of our survey, that was the limited number of patients with CVD and control group that were included in it.

Conclusion

CVD seems to be due to alteration of levels of trace elements in serum of patients, in this specificity, the significant increase of copper, and decrease in zinc and selenium

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