Study of Biochemical Levels In Serum And Saliva of Zinc and Copper in Patients With Stomatognathic System Dysfunctional Syndrome Following Bone Injury and Prosthetical Treatment

LAURA ELISABETA CHECHERITA, DANIELA TRANDAFIR*, OVIDIU STAMATIN, ELENA MIHAELA CARAUSU

1 Grigore T. Popa University of Medicine and Pharmacy, Department of Odontology-Periodontology and Fixed Prosthesis, 16 Universitatii Str., 700115, Iasi, Romania
2 Grigore T. Popa University of Medicine and Pharmacy, Department of Oro-Maxillo-Facial Surgery, 16 Universitatii Str., 700115, Iasi, Romania
3 Grigore T. Popa University of Medicine and Pharmacy, Department of Oral Implantology, 16 Universitatii Str., 700115, Iasi, Romania
4 Grigore T. Popa University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Management and Public Health Dentistry, 16 Universitatii Str., 700115, Iasi, Romania

Zinc (Zn) is abundant in bone tissue and is needed to maintain bone mineral density and bone metabolism. Taking into consideration the antagonism between Zn and copper (Cu), it is appreciated that the biochemical levels in serum and saliva of any of them, as well as in the Zn/Cu ratio are fundamental in the homeostasis of these trace elements in patients with bone injury and Stomatognathic System Dysfunctional Syndrome (SSDS). In the present study we are concerned if at patients with SSDS following fractures of mandible or of zygomatic-maxillary complex, are there any significant differences on serum or salivary biochemical levels of Zn and Cu, compared to controls consisting of healthy subjects. Even though in the cases groups there were revealed differences of the serum biochemical levels as well as in the saliva for Zn and Cu, compared to the control group, these differences did not prove to be statistically significant (p>0.05).

Key words: Stomatognathic System Dysfunctional Syndrome-SSDS, public health dentistry, divalent cations, serum, saliva, prosthetical treatment

The Zn present in the secretory granules of the salivary glands is also essential for the contraction of the myoepithelial structures of these glands. Zinc deficiency is accompanied by salivary secretion disorders and taste disorders [9]. Zinc deficiency and decreased Zn/Cu ratio in serum were identified as determinant factors of the immunity decrease and, as a consequence, as risk factors of various types of infection [10].

In patients with different types of cancer, including the oral cancer, the serum level of the Zn, as well as the ratio between the Zn concentration and the Cu is frequently low [11].

Copper is an essential element, which plays an important role in the production of haemoglobin, myelin and nerve function, bone growth, metabolism of the carbohydrates, catecholamines biosynthesis and formation of the cross-links between the collagen and elastin. It is a critical functional co-factor of important enzyme systems including the Cu center of cytochrome C oxidase. These enzymes are copper-enzymes [11].

The intake of Cu is primarily realized through diet; its absorption is done with ease, but it is limited by the homeostatic mechanisms after reaching the required. After absorption, Cu is bound to albumin and amino acids and in the blood Cu is found mostly in the form of ceruloplasmin. The Cu is stored in the liver and less in the kidneys. The main route of Cu elimination is biliary and then through the bowel, urinary excretion being reduced [12].

Copper is a strong antioxidant. Many important oxidation-reduction reactions in the body are catalysed by Cu [13]. It works together with an antioxidant enzyme, superoxide-dismutase (SOD), to protect cell membranes...
from being destroyed by free radicals. Copper is needed to make adenosine triphosphate (ATP), which is required to run the body.

Not only the concentrations of the Zn and Cu in the serum or saliva are important for the development of certain pathologic conditions, but also the ratio between them [14]. One of the most common trace-metal imbalances is elevated Cu and depressed Zn [15]. The Zn/Cu ratio is clinically more important than the biochemical levels of either of these trace metals.

**Experimental part**

The Stomatognathic System Dysfunctional Syndrome (SSDS) is the third disease of the Dental Medicine to be considered a public health problem due to its widespread prevalence and chronicity [16]; SSDS prevalence increases from 10% in the adults to almost 40% at people of 65 and over [17].

The purpose of this study is to check whether the presence of some Zn and Cu variations in SSDS patients determined by the affection of bone integrity by mandible fractures or by zygomatic-maxillary complex can constitute a valid argument which medically justifies the decision of administrating mineral supplements immediately post-fracture.

In the present study we are concerned if in patients with SSDS, following mandibular fractures or zygomatic-maxillary complex, there are statistical significant differences regarding serum or saliva biochemical levels of Zn and Cu, compared with a control group consisting of healthy subjects.

In order to reach the proposed aim, the present study has fixed the following objectives:
- determination of serum biochemical levels for Zn and Cu and of the serum Zn/Cu ratio in the study groups included;
- determination of saliva biochemical levels for Zn and Cu and the saliva Zn/Cu ratio;
- testing the statistical significance of the differences noted regarding the variation of the biochemical parameters analysed and the main demographic characteristics (gender and age) of the subjects in the study groups;
- emphasizing the eventual correlations (by calculating the “r” correlation coefficients) between the variation of the biochemical parameters analysed and the main demographic characteristics of the subjects in the study groups.

**Materials and methods**

Our study respected the methodology of the case-control studies.

Out of 615 cases of mandible fractures and 173 cases of zygomatic-maxillary complex fractures reported in 2015 for the area of Moldavia, there was chosen a representative group. In the study group 1 (cases group) there were include patients with SSDS, as a result of the damaging of the bone integrity by mandible fractures or of zygomatic-maxillary complex, admitted in the Oro-Maxillo-Facial Clinic at the St. Spiridon University Hospital of Iasi, in 2015.

In the cases group there were admitted subjects who simultaneously met the following conditions:
- minimum age of 18 years old;
- existence of the written informed consent regarding the participation in the study;
- harvesting of the biological samples (blood or saliva) could be realized prior to any therapeutic intervention;
- collecting of the biological samples could be realized at minimum 6 hour and maximum 48 hours from the trauma which affected the integrity of the stomatognathic system.

There were not admitted in the cases group:
- persons with affections that may interfere with the Zn and Cu homeostasis;
- patients who were administered drugs containing minerals or which significantly change the homeostasis of Zn and Cu, 4 weeks prior to the participation in the study;
- patients who were administered diuretic medication 4 weeks prior to their participation in the study (diuretic medication increases urinary Zn excretion by as much as 50%; prolonged use of diuretics could deplete Zn tissue levels, so clinicians should monitor Zn status [18]).

After fulfilling simultaneously the inclusion criteria, the cases group was comprised of 69 patients, including 22 females and 47 males. The average age in the cases was 48.07 ± 16.35 years, the minimum age was 19 years and the maximum of 79 years; the average age for males was 48.12 ± 16.30 years and 47.94 for female ± 17.02 years.

Of the total number of cases considered in the study, it can be noticed that the most frequent localization of the fracture was the mandibular one (52 cases), followed by the zygomatic-maxillary complex one (17 cases). For each case, the initial diagnosis was based on clinical history, physical examination, the correct diagnosis being radiology confirmed. In all the cases the diagnosis was made according to clinical criteria, radiological and laboratory.

In this study we respected the rules of GCP (Good Clinical Practice) [19].

The control group included adult volunteers who have expressed their consent freely on their participation in the study. In the control group there were included 43 healthy subjects, of which 28 females and 24 males. The average age in the control group was of 50.95 ± 18.13 years, the minimum age was 18 years and the maximum of 78 years. The average age for male gender was 56.47 ± 17.87 years and 46.58 for female ± 17.47 years. The proposal of participating in the controls was submitted to persons who have been to the dental office.

The structure of the study groups on the main demographic characteristics has revealed a good correspondence between them.

The collection of biological material (saliva and blood) in order to dose the Zn and Cu was realize before any therapeutic intervention addressed to the basic affections (12 h from the last meal, between 7-8 ante-meridian, a-juen) [20].

At the subjects considered, the saliva was harvested through the Holmes method which consists of aspiration (for 5 min). The method permits the collection of 1-2 mL of saliva. The saliva samples were centrifuged for 10 min, at 1400 rotations/min, after which the supernatant was painted in clean tubes at rates that were kept in a freezer (at -20°C) up to the time of actual measurements.

Biochemical levels in serum and saliva of Zn and Cu were established using the same protocol and method, also for the healthy subjects from the controls, but also for the SSDS patients determined by mandible fractures or zygomatic-maxillary complex.

For the analysis of the biological samples of serum and saliva in order to dosage the levels of Zn and Cu it was used, as a method, the spectrometry with atomic absorption (AAS) [21]. Dosages were conducted in an accredited medical laboratory.

For the statistical processing of the data it was generated a database using Microsoft Excel 2010 for Windows, and for the computerized statistical processing it was used SPSS 18.0 for Windows. With the help of the descriptive statistics module there were calculated the main statistical
indicators: medium value, standard deviation and the confidence interval (CI 95%). In order to check the statistical significance of the differences noticed, there were used tests of statistical significance ("t" Student test). By calculating the Pearson r correlation coefficients it was revealed the kind of interdependence between the studied variables and the intensity of the respective correlation. In the statistical processing of the data there were also considered the variability factors [22].

Ethical clearance for the study was obtained from the institutional ethical committee.

Results and discussions

Copper and Zinc are essential trace elements for the human body due to the multitude of functions in which they are involved and by the serious consequences on the body in case of shortage or deterioration of their activities and of the appropriate metalloenzymes.

Study of the serum concentrations of the Zn, Cu and the Zn/Cu ratio

In the first part of this article we were presenting the data referring to the serum levels of the Cu and Zn. There were measured the main indicators in order to evaluate their serum levels in the two groups considered in the study, the results being presented in table 1 comparatively.

From the results presented in table 1 it can be noticed that:

- even though in cases with SDSS due to mandible fractures or zygomatic-maxillary complex there are differences regarding the serum levels of the studied cations (of -3.09% for Zn and of +0.73% for Cu) compared to the controls, these differences did not prove to be statistically significant (p>0.05);
- also, there were not emphasized significant statistical differences regarding the serum Zn/Cu ratio in patients with SSDS vs. the controls.

Although there were also noticed relatively reduced values of the serum Zn (7.041 μg/dL) in a few cases with febrile state and sepsis, these values were not excluded from the beach set points. The Zn deficit is usually accompanied by a decrease in its excretion (urinary, salivary); however, the reduced serum values may also be correlated with urinary loss which may appear in chronic kidney and liver affections (cirrhosis) associated to the disease in the study.

The concentration of serum Cu reflects both the Cu bound to the ceruloplasmin, as well as the free Cu bound weaker to the albumin or to the small circulating peptides.

Ceruloplasmin is an acute phase reactant and because this protein binds a large amount of Cu, both the serum Cu, as well as the ceruloplasmin, grow in pathological conditions associated to inflammation. On the other hand, serum Cu levels may be low in conditions affections accompanied by hypoproteinemia (malnutrition, malabsorption), without necessarily reflecting reduced hepatic Cu deposits.

Study of saliva biochemical levels of Zn, Cu and saliva Zn/Cu ratio

In the second part of the article we presented the data regarding salivary concentrations of the Cu and the Zn. There were calculated the statistical indicators for their salivary levels in the two groups considered, the results being presented comparatively in table 2.

Analyzing the results synthetically presented in table 2, we can notice that:

- even though in cases of SSDS determined by mandible fractures or zygomatic-maxillary complex there are differences regarding the saliva's biochemical values of -6.30% for Zn and of +1.07% for Cu compared to the controls, these differences did not prove to be statistically different.

Correlation study

In order to measure the interdependence rate between the serum and saliva concentrations of the Zn and Cu and the different demographic variables investigated (at the controls vs. the cases with SSDS and mandible fractures or zygomatic-maxillary complex), there was calculated the statistical Pearson r correlation coefficient. The
interpretation of the $r$ correlation coefficient values was made according to the classic grid [23].

The Pearson $r$ correlation coefficient reveals a relatively strong negative correlation ($r$ correlation coefficient of -0.78), statistically significant ($p<0.05$), between the serum levels of Cu and the serum Zn/Cu ratio in the group of patients with SDSS determined by mandible fractures or of zygomatic-maxillary complex.

Our results also reveal medium negative correlations ($p<0.05$), in feminine gender, between age and the salivary concentration of the Zn ($r$ correlation coefficient of -0.40), Cu ($r$ correlation coefficient of -0.42) and of the Zn/Cu ratio in the saliva ($r$ correlation coefficient of -0.32), and between age and salivary concentration of the Cu ($r$ correlation coefficient of -0.24) in masculine gender.

Zinc is an essential transition metal in humans, playing a catalytic, structural and regulatory role in the biological system. Zinc is abundant in bone tissue and is needed to maintain bone mineral density and bone metabolism [24].

The results of our study have shown that in patients with SDSS due to mandible fractures or of zygomatic-maxillary complex there are no statistically significant differences regarding the serum and salivary concentrations of the Zn and Cu compared to the control group, which was formed of healthy volunteers.

In other studies [25], possible variations of the serum and salivary levels of the Zn and Cu compared to the controls were seen as a predisposing condition, and not as a consequence of the development of a pathological state in the investigated cases; in our study, the possible variations of the serum and salivary concentrations of the Zn and Cu compared to the controls were seen as a consequence of the fracture, because Zn is involved in the stimulation of bone formation by osteoblasts and inhibition of bone resorption by osteoclasts [26].

The studies in the detection of possible variations of Zn and Cu in patients with fractures targeted patients with fractures treated conservatively in which the traumatic context was less severe [27]. The present study took into account exclusively cases in which the traumatic context of producing the fracture was clearly spelled out, and the fragility of the mandible bone or zygomatic-maxillary complex could not be a factor to consider as predisposing of the occurrence of fracture [28].

In patients with severe trauma, there can be also seen significant changes if the serum levels of the Zn and Cu [29]. Mostly, this fact is attributed to the acute phase, characterized by an increase in the cytokine secretion, especially of interleukine 1 [30]. In this pathological complex, the stress answer is characterized, among others, by increased serum levels of the ceruloplasmin, C-reactive protein, but also by a decrease in the serum concentration of albumin, the protein involved in the transport and metabolism of the mentioned [31]. Moreover, a recent study proved a decrease of the serum concentrations of the Zn in patients with severe trauma (up to an average of 40.2±3.2 mg/L, compared to the normal interval considered of 70–120 mg/L) [32].

On the other hand, in the scientific literature there are also data which affirm the lack of changes in the serum levels of the Cu in the first 24 hours after the trauma, but because the cases have been monitored for a longer period of time there were found arguments for the increase of the serum level of the Cu starting with the 10th–11th day since the trauma took place. Because our study focused on the first 48 hours from the production of the trauma, we did not notice such changes.

Fracture healing involves complex processes of cell and tissue proliferation and differentiation. Many nutrients and other factors are involved, including hormones, growth factors, inflammatory cytokines, bone breakdown cells (osteoclasts) and bone building cells (osteoblasts), antioxidants, amino-acids [33]. Supplementation with Zn aids in callus formation, enhance bone protein production, and thus stimulates fracture healing.

Copper aids in the formation of bone, collagen and is important to the bone healing process. The body’s demand for both Zn and Cu rises according to the trauma severity [34].

Bone is a complex, living tissue which constantly changes and adapts itself to the demands put upon it. A myriad of nutrients, hormones, and other biochemical factors are essential to bone formation and maintenance, so an equal number of factors figure in the bone repair process.

In this type of Cases a prosthetic treatment was established at 75.36% of the cases. The patients received treatment by fixed prosthetic means, conjunct in 34.78% of cases and the remaining received mixed treatments, fixed and mobile at the rate of 42.02%, the rest of removable dentures 24.64%, thus restoring the morphological and functional the dental arches, thereby restoring the homeostasis of the stomatognathic system.

The subject concerning the level of Mg was studied in [35].

Conclusions

In cases with SDSS generated by mandible fractures or zygomatic-maxillary complex were emphasized differences of the serum biochemical levels (of -3.09% for Zn and of +0.73% for Cu) as well as the salivary biochemical levels (of -6.30% for Zn and of +1.07% for Cu) of the divalent cations studied, compared to the control group formed of healthy subjects, but the differences did not prove to be statistically significant ($p>0.05$). Thus, these variations do not medically justify the administration of mineral supplements in the cases of medium severity considered in the study, in the first 48 hours post-fracture.

Conflicts of interest

The authors declare that they are not in any kind of conflict of interests.

References

1. NRIAGU, J. Zinc Deficiency in Human Health, Office of Dietary Supplements, National Institutes of Health, Bethesda, Maryland 20892 USA.
2. KAUR, K., GUPTA, R., SHUBHINI, S., SHAILENDRA, SARAFK. Comprehensive Reviews in Food Science and Food Safety, 2014, 13, p.359-76.
15. KASLOW, J.E. Copper/Zinc Imbalance, Medical Board of California.
33. BROWN, S. Center for Better Bones, 605 Franklin Park Drive East Syracuse, 13057 NY.

Manuscript received:11.01.2015