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Concentrations of selected bioelements in saliva by patients suffering from macular degeneration related to age

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ABSTRACT

Aim of the study: Evaluation of zinc, copper and magnesium concentrations in the saliva of patients suffering from age-related macular degeneration (AMD).

Material and methods: The study was conducted amongst 151 patients of both sexes. The study group (B) consisted of 74 patients with AMD (BS – dry form, 53 people, BM – wet form, 21 people. The control group K consisted of 77 healthy patients. The diagnosis of AMD was based on the results of fundus examination, fluorescein angiography and OCT.

The material for biochemical tests was unstimulated mixed saliva. The measurement of the selected bioelements concentrations was made by atomic absorption spectrophotometry.

Results: The concentration of magnesium in the saliva was significantly higher in the study group compared to the control group (p = 0.0118). The concentration of zinc in the study group was significantly lower compared to the control group (p = 0.0429). In the case of copper concentrations, there were no differences between the test group and the control group.

Conclusions: Monitoring AMD patients and prophylaxis of healthy people at risk can be based on measurements of zinc and magnesium concentrations in saliva, giving the possibility of early detection of eye retinal anomalies and planning of diagnostic and therapeutic activities.

KEY WORDS: bioelements, saliva, macular degeneration related to age.

INTRODUCTION

An important etiological factor of age-related macular degeneration (AMD) is the inadequate response of the body to oxidative stress [1]. It involves the imbalance between processes generating reactive oxygen species (ROS) and enabling their utilization. One of the consequences of such a situation is the acceleration of cell ageing through the oxidation of their basic components: proteins, lipids, nucleic acids and carbohydrates. An important role in the system of protection of the body against the effects of ROS activities is played by some of the bioelements, including zinc, selenium, copper and magnesium. They take part in the operation of antioxidant systems, including enzymes such as superoxide dismutase and glutathione peroxidase.

Zinc is mostly located in the intracellular space, where it occurs in the ionized form. Some of the zinc ions are associated with a specific carrier protein – zinc metallothionein – and some are part of many enzymes involved in the synthesis of nucleic acids. Zinc supplementation may prevent the reduction of insulin secretion and adrenocortical activity, as well as increasing the body's resistance [2-4]. However, excessive supply of zinc in the diet may impair the metabolism of calcium, copper and manganese [5, 6].

Copper is an essential micronutrient needed for the proper development and operation of the human body, which along with zinc is on the list of supplements recommended in AMD therapy. The basic role of copper is connected with oxidation and reduction processes. Its deficiency can lead to disorders in connective tissue, depigmentation or disorders in the development of blood vessels, among others. Excess copper is just as dangerous as its deficiency. It can stimulate the formation of ROS.

The bioelement for normal homeostasis of the human body is magnesium, classified in the group of macronutrients [7]. The relationship between magnesium deficiency and cardiovascular diseases, mainly hypertension, is known as

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the main AMD risk factor. Most intracellular magnesium is found in the mitochondria, where it is associated with ATP, while ionized magnesium is found mostly in the cytosol, where it acts as an activator of more than 300 different enzymes and participates in regulation of many metabolic pathways. In the last years, stressogenic methods of obtaining biological material for bioelement analyses (injection into a vein) are being successfully replaced by a non-invasive analysis method taken from saliva [8]. As research material, saliva does not lose its properties for a week if it is stored at 4 C. Another advantage is that it can be taken even several times a day if it is necessary monitor some parameters or drug levels.

AIM OF THE STUDY

1. Evaluation of zinc, copper and magnesium concentrations in the saliva of patients suffering from age-related macular degeneration (AMD).

2. Comparative analysis of concentrations of the analyzed bioelements in saliva including AMD figure.

The research procedure was approved by resolution No. KB-0012/72/12 of the Bioethical Committee of the PUM in Szczecin.

MATERIAL AND METHODS

Analysis of zinc, copper and magnesium concentrations in saliva was carried out in 151 people, 94 women aged from 40 to 87 years (average 66.8 years) and 57 men from 48 to 84 years (average 68.5 years). Recruitment for the study was carried out amongst patients diagnosed with AMD, under the care of the PUM Ophthalmology Clinic in Szczecin, participants of the meetings of the Szczecin Branch of the Association for AMD, and amongst the ophthalmologically healthy patients of the dental office. The study group (B) consisted of 74 AMD patients, including 48 females aged 40-87 (average 70.3 years) and 26 men aged 49-84 (average 70.7 years). The control group (K) consisted of 77 ophthalmologically healthy patients, including 46 women aged from 48 to 80 years (average age 63) and 31 men aged from 48 to 80 years (average 66.5 years). In the study group (B) two subgroups of patients were distinguished taking into account the disease form: a) BS (dry form) – 53 people (34 women and 19 men) and b) BM (wet form) – 21 people (14 women and 7 men). The diagnosis of AMD was based on the results of: fundus examination, ophthalmoscopy, fluorescein angiography (AF) and coherence tomography (OCT). The material for biochemical tests was unstimulated mixed saliva, which the patients spit for 15 minutes into plastic tubes. Those qualified for the study were asked to refrain from taking meals, drinks, mouthwash and smoking at least an hour before the start of the study. On the day before the examination, the patient's diet did not differ from the one used so far. Before the saliva collection, the tested participants had to undergo a dental examination. The collection of saliva was carried out at the same time of the day – in the morning from 8.00 to 11.00. The material collected in the test tubes was placed in an ice container and sent immediately to the laboratory of Biochemistry and Medical Chemistry department of PUM, where it was frozen down to –20°C and stored until analysis time. After thawing, the saliva was centrifuged for 15 minutes at 4000 rpm and then diluted with TISAB II in a 1:1 ratio and distilled water in a ratio of 0.5 ml saliva/5 ml water. Magnesium concentrations were measured in such diluted samples. Measurements of zinc and copper concentrations were performed in such centrifuged saliva diluted with TISAB II buffer and then diluted three times with distilled water. Measurements of concentrations of the analyzed bioelements were carried out by atomic absorption spectrophotometry using a Philips 9100X atomic absorption spectrometer, calibrated using standard solutions. For magnesium, a working wavelength of 285.2 nm was used, for zinc 213.9 nm, for copper 354.8 nm. The determinations were made in an acetylene-oxygen flame. In the determination of magnesium concentrations, a 0.5% solution of lanthanum nitrate (Lanthannitrat Hexahydrat from Merck) as an ionization buffer was additionally used. The readings of bioelement concentrations were made from the Merck Titrisol standard curve. All continuous variables were checked for normality of distribution using the Kolmogorov-Smirnov test. These variables are described by average values and standard deviations as well as the minimum and the maximum values. Checking the statistical significance of the differences between the two groups was tested using Student's test and the Mann-Whitney U test. For many groups, the analysis of variance (ANOVA) or covariance (ANCOVA) or Kruskal-Wallis test was used. Pearson's χ^2 test was used to examine statistical relationships between discontinuous variables. The results were presented by specifying the correlation coefficient r and probability p. The statistically significant differences in all performed tests were those for which the probability was $p < 0.05$. Statistical analyses were performed using the STATA 11 statistical program (StataCorp, USA); license number 30110532736.

RESULTS

Magnesium

The average concentration of magnesium in the saliva of the tested subjects from the study group without taking into account the division into subgroups was significantly higher compared to the values recorded in the control group ($p = 0.0118$). Taking into account the division into subgroups, it was found that in both the subgroup of patients with dry form and those with wet form, these concentrations were higher than in the control group, but statistical significance of the difference was demonstrated only in the case of people suffering from dry AMD ($p = 0.0139$). There was no difference between the study subgroups. Detailed data on magnesium concentration measurements are presented in Tables I, II and III.

Zinc

The average concentration of zinc in the saliva of subjects from the study group without considering the division

Table I. Concentration of magnesium in the saliva of people from the test group compared to the control group [mg/l]

Group	*N*	Mg Average	Mg SD	Mg Min.	Mg Max.	Mg Q25	Mg Median	Mg Q75	*p*
K	77	4.00	1.98	1.00	9.66	2.66	3.56	4.84	0.0118
B	74	5.13	3.29	1.27	16.26	2.64	4.13	6.36	

Mg – magnesium; K – control group; B – study group

Table II. Concentration of magnesium in saliva depending on the form of AMD compared to the control group [mg/l]

Group	*N*	Mg Average	Mg SD	Mg Min.	Mg Max.	Mg Q25	Mg Median	Mg Q75
K	77	4.00	1.98	1.00	9.66	2.66	3.56	4.84
BS	53	5.19	3.41	1.27	16.26	2.56	4.24	6.42
BM	21	4.97	3.03	1.99	13.80	3.05	3.96	6.36

Mg – magnesium; K – control group; BS – dry form; BM – wet form

Table III. Statistical analysis of differences between the test and control subgroups and between the two study subgroups

Groups compared	Dependent variable	Student's t-test	*p*	Number N1	Number N2
K	BS	Mg	0.0139	77	53
K	BM	Mg	0.0824	77	21
BS	BM	Mg	0.8019	53	21

Mg – magnesium; K – control group; BS – dry form; BM – wet form

into subgroups was significantly lower compared to the values recorded in the control group ($p = 0.0429$). Considering the subgroup division, it was found that in both the subgroup of patients with dry form and those with wet form, these concentrations were lower than in the control group, but the statistical significance of the difference was demonstrated only in the case of people suffering from dry AMD ($p = 0.0490$). There was no difference between the study subgroups. Detailed data on zinc concentration measurements are presented in Tables IV, V, VI and Figure 1.

Copper

The average concentration of copper in the saliva of from the study group without taking into account the subgroup division did not differ from the average concentration recorded in the control group ($p = 0.8993$). Considering the division into subgroups, there were no differences when compared to the control group. There was also no difference between the study subgroups. Detailed data on zinc concentration measurements are presented in Tables VII, VIII, IX and Figure 2.

Correlation of concentrations of individual bioelements

For the whole population of patients, there was a significant positive correlation between the concentrations of zinc and copper ($p = 0.0021$). There was also a significant positive correlation between the concentrations of zinc and copper in the study group without considering the division into subgroups ($p = 0.0025$), as well as in the subgroup suffering from dry AMD ($p = 0.0113$) (Table X).

DISCUSSION

There is a natural antioxidant system in the body that protects cells from the adverse effects of reactive oxygen species (ROS). It consists of non-enzymatic antioxidants and antioxidant enzymes. Two of these enzymes – superoxide dismutase (SOD) and glutathione peroxidase (GPx) – require the presence of cofactors in the form of bioelements supplied in the daily diet. The excessive presence of oxygen in the microenvironment of the retinal photoreceptors promotes the formation of ROS. Their overproduction causes destructive changes within the photoreceptor complex – retinal pigment epithelial cells. In addition, damage to the photoreceptor cell membrane, especially its outer layer containing a lot of polyunsaturated fatty acids, progresses as a result of the acceleration of fatty acid oxidation and the activation of the cytotoxic chain reaction [9].

A properly balanced diet, rich in antioxidant substances, is of great importance in the prevention and treatment of AMD. However, when using a diet that is deficient in terms of antioxidant content, an important role is played by supplements that could supplement such deficiencies. That is

Table IV. Concentration of zinc in the saliva of people from the test group compared to the control group [mg/l]

Group	*N*	Zn Average	Zn SD	Zn Min.	Zn Max.	Zn Q25	Zn Median	Zn Q75	*p*
K	77	0.55	1.34	0.03	11.30	0.17	0.24	0.44	0.0429
B	74	0.30	0.32	0.10	2.04	0.15	0.20	0.33	

K – control group; B – study group

Table V. Concentration of zinc in saliva depending on the form of AMD compared to the control group [mg/l]

Group	*N*	Zn Average	Zn SD	Zn Min.	Zn Max.	Zn Q25	Zn Median	Zn Q75
K	77	0.55	1.34	0.03	11.30	0.17	0.24	0.44
BS	53	0.29	0.29	0.10	2.01	0.14	0.20	0.33
BM	21	0.32	0.40	0.11	2.04	0.17	0.20	0.32

K – control group; BS – dry form; BM – wet form

Table VI. Statistical analysis of differences between the test and control subgroups and between the two study subgroups

Groups	Dependent variable	Mann-Whitney test	*p*	N1	N2
K	BS	Zn	0.0490	77	53
K	BM	Zn	0.2928	77	21
BS	BM	Zn	0.5407	53	21

K – control group; BS – dry form; BM – wet form

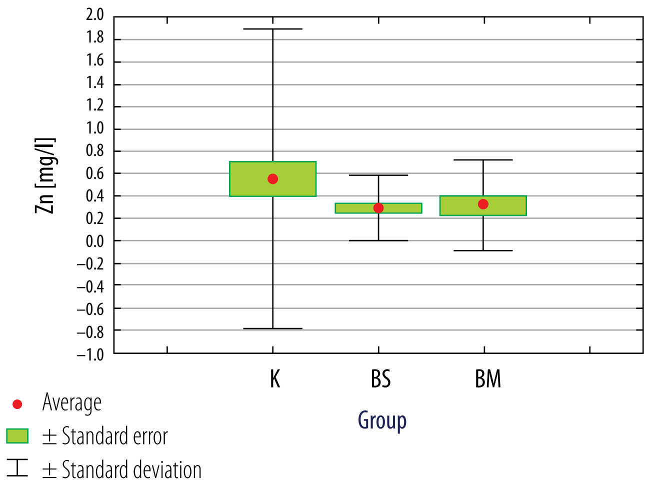

Figure 1. Concentration of zinc in saliva depending on the form of AMD as compared to the control group

why many of the previous activities have focused on creating the perfect composition of dietary supplements. For example, in randomized studies AREDS was administered to patients for 5 years AREDS set, which contains vitamins and bioelements, including zinc (80 mg in the form of zinc oxide) and copper (2 mg as copper oxide) [2]. The progress of degenerative changes in the macula in groups with early and moderate AMD was slowed. The risk of subretinal neovascularization was also reduced and the potential transition time to the advanced form was increased by approximately 30%. It was estimated that among patients with AMD who used dietary supplements, the risk of the disease becoming more advanced in five years was about 1.3% [2].

In the cited studies of AREDS and many similar research projects, ophthalmologic changes were evaluated after using specific vitamins and bioelements. However, their concentrations in biological fluids were not analyzed, taking into account the control group [10]. However, since the correlation between the presence of antioxidants in the diet and the stage of AMD has been confirmed, and the effect of increased doses of antioxidant substances on the slowing down of the disease process has been proven, the question arises about the correlation between the concentration of bioelements supporting antioxidative processes in the human body and the risk of developing AMD.

The elements analyzed in this study are indispensable components of the daily diet. They are part of, among others, antioxidant enzymes. Their proper content in the body determines the proper course of enzymatically catalyzed biochemical reactions, in which these elements act as cofactors. Zinc and copper play an extremely important role in the proper functioning of the retina, through participation in the regulation of antioxidative processes [11, 12]. They belong to the main components of commonly used multivitamin preparations taken by patients [13, 14]. Bearing in mind the complex etiology of AMD, it was considered

Table VII. Concentration of copper in the saliva of people from the test group compare to the control group [mg/l]

Group	*N*	Cu Average	Cu SD	Cu Min.	Cu Max.	Cu Q25	Cu Median	Cu Q75	*p*
K	77	0.27	0.13	0.07	1.00	0.21	0.23	0.30	0.8993
B	74	0.27	0.15	0.06	1.12	0.19	0.24	0.29	

K – control group; BS – dry form; BM – wet form

Table VIII. Copper concentration in saliva depending on the form of AMD compared to the control group [mg/l]

Group	*N*	Cu Average	Cu SD	Cu Min.	Cu Max.	Cu Q25	Cu Median	Cu Q75
K	77	0.27	0.13	0.07	1.00	0.21	0.23	0.30
BS	53	0.27	0.14	0.15	1.12	0.20	0.24	0.29
BM	21	0.27	0.19	0.06	1.00	0.19	0.23	0.27

K – control group; BS – dry form; BM – wet form

Table IX. Statistical analysis of differences between the test and control subgroups and between the two study subgroups

Groups	Dependent variable	Mann-Whitney test	*p*	N1	N2
K	BS	Cu	0.7962	77	53
K	BM	Cu	0.4434	77	21
BS	BM	Cu	0.4148	53	21

K – control group; BS – dry form; BM – wet form

reasonable to investigate the concentrations of these elements in saliva. In the population of patients diagnosed with AMD qualified for this study, the concentrations of zinc, copper and magnesium in saliva were measured with the intention of comparing them with the concentrations recorded in the control group. It was found that the average concentration of zinc in the control group was significantly higher than in the group of patients (Table IV). This result may indicate the existence of larger resources of this element in the body of healthy people, which provides them with more effective antioxidant mechanisms, and thus enables more effective protection against the occurrence of AMD symptoms. This is confirmed in the comparison of the control group with the subgroup of patients with the AMD dry form, suggesting that zinc-dependent antioxidant protective mechanisms are found in patients with a functional limit (Table VI). It can, therefore, be assumed that these people have limited availability of zinc for antioxidant enzymes. According to Grahn *et al.*, this situation may favor the initiation of pathological changes in the retina [12]. Wills *et al.* believe that the concentration of zinc in the retina is even and comparable with the concentration in other tissues and body fluids, which can also be related to saliva [11].

Measurements of concentrations of bioelements in saliva are successfully used in the diagnosis of other diseases [15]. Ayinampudi *et al.* describe the possible use of zinc and copper concentrations in saliva as markers of pre-cancer and oral cancer [16]. The excess of zinc in the diet impairs the metabolism of calcium, copper, iron and manganese. For this reason, supplementation with large doses of zinc should be accompanied by copper supplementation [14].

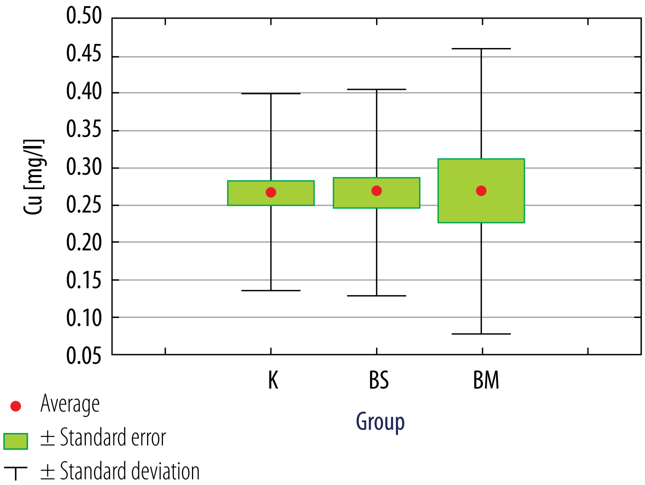

Figure 2. Copper concentration in saliva depending on the form of AMD in comparison to the control group

Copper is a cofactor of superoxide dismutase, an essential micronutrient in the body, whose concentration can be measured in saliva. Wills *et al.* emphasize the role of copper in the proper function of photoreceptors in the retina [11]. Together with zinc, it cooperates in antioxidant activity. Based on evidence from numerous reports, it seems to be a combined effect. It can be assumed that its effect is also limiting

Table X. Spearman's rank correlation values and their statistical significance

Variable pairs		*N*	R	*p*					
Mg	Zn	151	0.08	0.3551					
Mg	Cu	151	0.06	0.4749					
Zn	Cu	151	0.25	0.0021					
Variable pairs		**Group**	***N***	**R**	***p***	**Group**	***N***	**R**	***p***
Mg	Zn	K	77	0.06	0.6188	B	74	0.19	0.1130
Mg	Cu	K	77	0.04	0.7446	B	74	0.07	0.5600
Zn	Cu	K	77	0.14	0.2153	B	74	0.35	0.0025
Variable pairs		**BS**	***N***	**R**	***p***	**BM**	***N***	**R**	***p***
Mg	Zn	BS	53	0.26	0.0642	BM	21	0.01	0.9664
Mg	Cu	BS	53	0.04	0.7871	BM	21	0.19	0.4000
Zn	Cu	BS	53	0.35	0.0113	BM	21	0.39	0.0826

K – control group, BS – dry form, BM – wet form

the formation and development of AMD [17]. These conclusions are confirmed by the results of this research, indicating the existence of a significant positive correlation between the concentrations of copper and zinc in the tested saliva (Table X). A similar relationship was observed by Wills *et al.* [11].

Both the deficiency of copper and its excess in the body can lead to adverse effects. Characterized by a high redox potential, copper ions can be toxic to the cell and paradoxically promote the formation of free radicals that generate oxidative stress. According to Błoniarz *et al.*, the concentration of copper in saliva may be a marker of some diseases [18]. For example, in the saliva of patients with diagnosed squamous cell carcinoma of the oral cavity, increased concentrations of copper, but also of zinc, calcium, magnesium, sodium, manganese and iron, have been demonstrated.

When analyzing magnesium levels in the saliva of people surveyed in this study, attention should be paid to their much higher values compared to the concentrations of zinc and copper. Magnesium is the second most widespread cation in the intracellular space and one of the main macronutrients found in the human body. Fulgoni *et al.* mention magnesium as one of the basic components of dietary supplements [14].

In this study, significantly higher concentrations of magnesium in the saliva of people with AMD were noted than in the control group (Table I). These differences were mainly due to the values shown in the subgroup of patients with dry disease (Table II). It seems that the concentration of this bio-element in saliva may be a marker for the diagnosis of AMD, because in all forms of this disorder, values are higher than in healthy people.

The usefulness of the use of saliva in medical analytics to assess the concentration of bioelements is emphasized by many authors. The method is considered reliable and comparable to a blood test [19]. The research carried out in this work sought to demonstrate the desirability of measuring the concentrations of zinc, copper and magnesium in the saliva of patients diagnosed with AMD, especially since the literature is virtually devoid of reports on this subject. Due to their non-invasive nature, lack of complexity and low costs, they should find a place in routine diagnostics.

CONCLUSIONS

Monitoring AMD patients and protection of healthy people from risk may be based on measurements of zinc and magnesium concentrations in saliva, giving the possibility of early detection of eye retinal abnormalities and planning of diagnostic and therapeutic activities.

DISCLOSURE

The authors declare no conflict of interest.

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