Trace Element Levels in Blood Serum and Colon Tissue in Colorectal Cancer

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The analyses of Cu, Mg, Se, and Zn in blood serum and colon tissue samples from twenty colorectal cancer patients by atomic absorption spectrometry (AAS) were accomplished. Serum levels of these elements were compared with contents found in control group samples. The mean serum levels of Se were lowered in colorectal cancer compared to controls. The observed serum content of Zn was significantly higher in patients. Copper and magnesium serum contents did not significantly differ as compared to healthy population. The negative correlation ($R = -0.444$) between selenium levels in serum and colon tissue of cancerous patients has been found. The cancerous colorectal tissue showed content of Cu (6.08 ± 3.78) μg g^{-1}, Mg (753.59 ± 310.54) μg g^{-1}, Se (1.17 ± 0.73) μg g^{-1}, and Zn (69.20 ± 21.03) μg g^{-1}.

It is known that trace elements have an important influence as a component of many enzymes on a large number of biological processes. They have some regulatory functions and they may affect immune reactions and free radical generation. The extremely low contents of these trace elements and their diversity imply that their molecular effect is very specific and that they may not be substituted for one another.

The cancerous tissue directly indicates changes of trace elements that are evoked by the disease. Blood or blood constituents such as serum are considered to be the best indicators of the present exposure of an individual to many metals due to its easy sampling. We focused on four elements: copper, magnesium, selenium, and zinc. The parameters of atomic absorption spectrometry determination, sample preparation procedures, and the discussion of the obtained results are presented in this paper.

Selenium came into general knowledge as strongly toxic, but now it is considered as an essential element with several important roles in the human body. For example, it functions as an antioxidant with interrelationship with vitamin E, and component of the antioxidant enzyme glutathione peroxidase, what has been fully documented [1, 2]. Normal cells are protected by antioxidant enzymes from the toxic effects of high concentrations of reactive oxygen species generated during cellular metabolism. Even though cancer cells generate reactive oxygen species, it has been demonstrated biochemically that antioxidant enzyme levels are low in most human cancers [3]. Essential trace elements copper and zinc are micronutrients that play important roles in different biochemical reactions. These two metals are important for optimum function of the immune system and act as cofactors of Cu,Zn-superoxide dismutase (SOD) [4]. This enzyme, as essential part of the enzymatic antioxidant system, catalyzes the dismutation of the superoxide anion to hydrogen peroxide.

In recent years, metal contents in human body have been used as an aid for diagnosing various diseases including cancer [5]. We analyzed also magnesium, which also belongs to essential elements, in addition to the commonly analyzed selenium, copper, and zinc in the blood serum and tissue of patients with colorectal cancer. The aim of the study is to investigate the contents of trace elements in blood serum and the incidence of cancer.

EXPERIMENTAL

We investigated 20 blood serum and tumour tissue samples of patients with colorectal cancer from the Moravian region of the Czech Republic (obtained from Teaching Hospital in Olomouc). This group of patients consisted of 7 women (61—78 years old) and 13 men (between 60 years and 79 years old). The blood serum samples were taken in the same day as surgical intervention took place. The control serum samples were collected from 10 healthy volunteers consisting

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of 5 healthy women and 5 healthy men with matching age (60—70 years old). Samples were stored in plastic tubes at −20 ℃. Serum samples were defrosted near the room temperature and homogenized by stirring for about 30 s. Tissue samples were defrosted by similar way and then they were dried for the duration of 4 h at 65 ℃ in hot-air drying oven and weighed. Such drying temperature has been chosen to minimize the possible loss of the volatile elements, which can occur at temperatures higher than 60 ℃ [6]. After these procedures both types of samples were decomposed using the microwave digestion with concentrated nitric acid, 3 cm^3 HNO_3 for 1 cm^3 serum or 1 g dried tissue in microwave digestion unit BM-1S (Plazmatronika, Poland). The following program for the digestion has been used: 60 s power 50 %, 30 s power 0 %, 60 s power 60 %, 30 s power 0 %, 180 s power 80 %, 30 s power 0 %, and 360 s power 100 %, which has been followed by 10 min long cooling. The average weighed amount of dried tissue has been 0.588 g. After the mineralization nitric acid was evaporated and samples were completed with deionized water to defined volume.

The atomic absorption spectrometer GBC Avanta Σ equipped with flame atomization and hydride generation system HG 3000 with electric heating accessory EHT-10 (GBC, Australia) was used for all measurements. The instrument was equipped with a deuterium lamp background correction system. Standard instrument parameters for the analysis were applied. Copper, magnesium, and zinc were determined by the flame atomization technique with an acetylene-air flame. Selenium was determined using the hydride generation technique. The quartz absorption tube for hydride generation was heated to 950 ℃.

For the decomposition of samples superpure nitric acid (Carlo Erba Reagenti, Italy) was used. Calibration solutions were prepared from the stock solutions of (1.000 ± 0.002) g dm^{-3} (Analytika, Czech Republic). For hydride generation 0.6 % solution of sodium borohydride (approx. 98 %, Sigma, Germany) in 0.6 % sodium hydroxide (anal. grade, Lachema, Czech Republic) was prepared. All samples were prereduced in approximately 5 mol dm^{-3} hydrochloric acid (Analpure SD, Analytika, Czech Republic) at 85 ℃ for 30 min. All water designated to solutions and samples preparation was purified by demineralization (Elga system, Great Britain). Reference material Seronorm^{TM} Trace Elements Serum; batch 704121 (Nycomed Pharma AS, Norway) and certified reference material of pork liver GBW 08551 (Food Detection Science Institute, Ministry of Commerce, China) were used for accuracy control of the analysis.

RESULTS AND DISCUSSION

The detailed statistic treatment containing exploratory and confirmatory data analysis has been applied to all results. For the examination for normality of sample distribution the test of combined sample skewness and kurtosis has been used. The test for normality of sample distribution for Cu, Mg, and Se in blood serum of cancerous patients leads to the conclusion that the normality has been accepted. The normality for zinc in the serum and for copper in tissue samples is rejected. This test accepts normality for Mg, Se, and Zn in data obtained from the analysis of colon tissue. To compare the data, a Box—Cox transformation was used (to obtain Gaussian distribution), and re-expressed mean and standard deviation served as parameters of location and spread. Details of confirmatory data analysis are summarized in Tables 1 and 2.

Table 1. Results of Analyzed Elements in Serum of Cancerous Patients

Element	Cu	Mg	Se	Zn
Skewness	0.16	−0.84	0.23	1.56
Kurtosis	2.18	3.05	2.30	5.11
ρ(mean*)/(mg dm^{-3})	0.90	18.23	0.041	1.03
ρ(standard deviation)/(mg dm^{-3})	0.20	2.93	0.009	0.57
ρ(median)/(mg dm^{-3})	0.86	18.57	0.039	1.02

* For Cu, Fe, Mg, Se arithmetic mean, for Zn re-expressed mean after Box—Cox transformation.

Table 2. Results of Analyzed Elements in Colon Tissue of Cancerous Patients

Element	Cu	Mg	Se	Zn
Skewness	1.27	−0.03	1.03	−0.84
Kurtosis	5.44	2.11	3.55	3.08
w(mean*)/(μg g^{-1})	6.08	753.59	1.17	69.20
w(standard deviation)/(μg g^{-1})	3.78	310.54	0.73	21.03
w(median)/(μg g^{-1})	7.24	753.71	1.01	72.13

*For Fe, Mg, Se, Zn arithmetic mean, for Cu re-expressed mean after Box—Cox transformation.

A procedure recommended for statistical analysis of small samples (less than 20) based on order statistics [7] has been used to estimate parameters of location and spread for control group and sex matched patient's groups. The estimate of parameter of location in the control group is then expressed by the pivot polosum and its 95 % confidence interval. The obtained results for determined elements were for Cu 1.05 mg dm^{-3} with confidence interval 0.96—1.15 mg dm^{-3}, for Mg 18.43 mg dm^{-3} with interval 12.28—24.58 mg dm^{-3}. Pivot polosum for Se was 93.88 μg dm^{-3} and its interval 80.96—106.79 μg dm^{-3} and for Zn 0.82 mg dm^{-3} with interval of confidence 0.49—1.14 mg dm^{-3}. The assumption of normality in control group samples has been accepted for all studied elements. The results in patients group with regard to sex are presented in Table 3 for blood serum and Table 4 for colon tissue.

Table 3. Evaluation of the Results in Serum Based on Gender of Patients

	Men			Women		
Element	Pivot polosum	Confidence interval (95 %)	Median	Pivot polosum	Confidence interval (95 %)	Median
ρ(Cu)/(mg dm^{-3})	0.79	0.72—0.87	0.80	1.05	0.83—1.27	1.05
ρ(Mg)/(mg dm^{-3})	18.02	15.31—20.72	17.65	19.64	16.95—22.32	18.73
ρ(Se)/(μg dm^{-3})	37.42	27.98—46.85	37.57	45.89	36.93—54.84	47.05
ρ(Zn)/(mg dm^{-3})	1.02	0.87—1.16	1.04	1.02	0.66—1.38	1.00

Table 4. Evaluation of the Results in Colon Tissue Based on Gender of Patients

	Men			Women		
Element	Pivot polosum	Confidence interval (95 %)	Median	Pivot polosum	Confidence interval (95 %)	Median
w(Cu)/(μg g^{-1})	6.24	3.90—8.57	7.46	6.49	2.27—10.71	5.59
w(Mg)/(μg g^{-1})	825.03	607.65—1042.41	767.57	692.25	203.59—1180.91	586.29
w(Se)/(μg g^{-1})	1.22	0.69—1.75	1.21	1.07	0.21—1.93	0.69
w(Zn)/(μg g^{-1})	74.76	67.23—82.28	72.19	62.89	25.17—100.60	64.07

The accuracy of the elements determination in tissue and serum has been expressed in terms of recovery of spiked additions of each element. The average values of the results/% are included with the respective standard deviations (97.6 ± 4.2 and 87.6 ± 7.8 for Cu, 111.1 ± 3.9 and 96.2 ± 3.1 for Mg, 84.5 ± 9.6 and 88.2 ± 13.4 for Se, and 100.3 ± 2.0 and 101.6 ± 1.2 for Zn, in serum and tissue, respectively). The comparison between found and recommended values in reference material as arithmetic mean and sample standard deviation of three replicates are shown in Tables 5 and 6. Generally speaking, the measured values do not deviate significantly from the recommended or certified values. The obtained results from certified reference material of pork liver show that there is no significant loss of the volatile elements during drying of tissue samples. Precision of the Cu, Mg, and Zn determination in serum evaluated as relative standard deviation (RSD) of five replicates has been lower than 5 % and for Se it has been 12 %. The precision in colon tissue has not been evaluated because of the inhomogeneity of tissue samples. Limits of detection calculated by Kaiser's 3σ methods were in blood serum for Cu 7.3 μg dm^{-3}, for Mg 1.1 μg dm^{-3}, for Zn 5.9 μg dm^{-3}, and for Se 0.24 μg dm^{-3}. In colon tissue samples the limits of detection have been calculated for the average amount of dried tissue for Cu and Zn 0.3 μg g^{-1}, for Mg 0.1 μg g^{-1}, and for Se 0.04 μg g^{-1}.

The Student's tests ($1 - \alpha = 0.95$) have been used to determine statistically significant differences between the contents in the cancerous patients and control group of healthy people. If one of the two compared samples has not proved normality assumption (Zn), a robust Student's test has been calculated. The mean serum selenium level in the colorectal cancer group has been significantly lower than in the control group. Statistically evaluated levels of the p-value ($p < 0.05$) for zinc in serum lead to significantly higher concentration of this element in cancer-affected than in healthy subjects. In our study there have been no changes in serum copper and magnesium levels in colorectal cancer disease and control group. We assessed using the Student's test significantly increased levels of Se and Cu in serum of women in comparison with men ($p < 0.05$). There have not been found any differences between mean values of Mg and Zn in serum and all the analyzed elements in tissue of female and male samples. The serum level of $Cu_{content}/Zn_{content}$ ratio was lower in colorectal cancer patients (0.94 ± 0.43) than in healthy subjects (1.73 ± 0.61) (arithmetic mean ± standard deviation).

Table 5. Recommended and Found Concentrations in the Reference Material Seronorm^{TM}

Element	Recommended value	Found value*
ρ(Cu)/(mg dm^{-3})	1.3	1.2 ± 0.1
ρ(Mg)/(mg dm^{-3})	20	20.9 ± 0.8
ρ(Se)/(μg dm^{-3})	80	74.5 ± 2.5
ρ(Zn)/(mg dm^{-3})	1.48	1.4 ± 0.1

*Arithmetic mean ± standard deviation.

Table 6. Certified and Found Concentrations in the Certified Reference Material GBW 08551

Element	Certified value*	Found value*
ρ(Cu)/(μg dm^{-3})	17.2 ± 1.0	15.9 ± 0.6
ρ(Mg)/(μg g^{-1})	747 ± 52	708 ± 21
ρ(Se)/(μg g^{-1})	0.94 ± 0.05	0.86 ± 0.07
ρ(Zn)/(μg g^{-1})	172 ± 4	178 ± 6

*Arithmetic mean ± standard deviation.

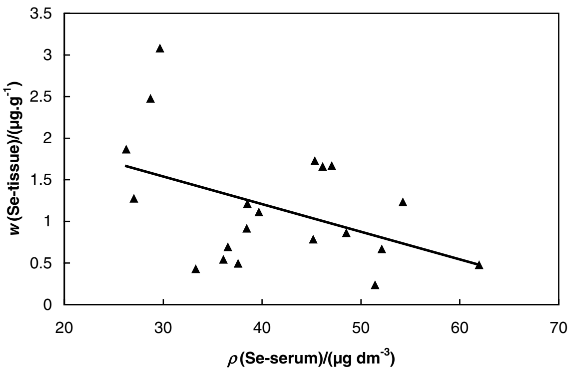

Fig. 1. Correlation between selenium in serum and tissue.

We have observed significant negative correlation (Person's $R = -0.444$, Spearman's rank correlation -0.341) between selenium levels in serum and colon tissue of cancerous patients (Fig. 1). On the other hand, there have been no correlations ($|R| < 0.1$) between concentration levels in serum and tissue of the other elements. An inverse correlation has also been found between serum and tissue levels of selenium in males (Person's $R = -0.392$, Spearman's rank correlation -0.319) and females (Person's $R = -0.385$, Spearman's rank correlation -0.571).

In the literature, there are controversial data about copper and zinc levels in cancerous patients. Here there are only a few examples. *Magálová* and her coworkers [4] did not find any changes in serum zinc levels in colon or rectal cancer and they proved significantly increased serum Cu level in rectal cancer, whereas in colon cancer it was not changed. Serum and tissue levels of Cu and Zn are presented in the study [8], where mean serum copper levels were higher in patients with colorectal cancer and the mean serum zinc levels were lowered only in advanced colorectal cancer compared to controls. Zn is proved to be an antioxidant or free-radical scavenger. Changes in Zn level in blood of cancerous patients may be the cause of malignant tumour occurrence. Review by *Navarro-Alacrón* and *López-Martínez* [9] observed significant decrease of serum or plasma Se levels in different types of cancer-affected humans. Selenium deficiency may be caused by the low Se content in foodstuff, which mostly contributes to the intake of Se by human. The anticancer effect of Se has been supported by animal experiments, human epidemiological investigation, and intervention trials [10]. However, the mechanism of Se as an anticancer agent is not clear yet, further research, especially in individual selenoproteins, is needed to understand the function of selenium in each of the cell types of the immune system.

Because of the lack of control group samples of colon tissue, it is possible to compare our results only with some literature data. The cancerous colorectal tissue presented by *Gupta* and coworkers [8] showed the following contents: 2.78 μg Cu in g and 27.16 μg Zn in g, our results for copper and zinc are two or three times higher. The work [11] shows approximately five times lower concentrations for Cu and Zn in colorectal tissue than are presented in our work. The authors of that study coming from the beginning of the eighties have developed X-ray fluorescence method for seven trace elements. Comparative analysis of selenium in human colon has been carried out [12] with the determined Se contents (0.07 ± 0.02) μg g^{-1} and (0.05 ± 0.01) μg g^{-1} for aqueous calibration and standard additions, respectively. Magnesium is not commonly determined in colon tissue, we have not been able to compare it with published data.

CONCLUSION

The microwave-assisted digestion and atomic absorption determination of Cu, Mg, Se, and Zn have proved to be simple and effective way to find contents levels of these elements in blood serum and colorectal tissue. The results presented in this work show statistically significant changes in Se and Zn levels in serum of colorectal cancer patients in comparison to control group. The significant negative correlation between the levels of Se in blood serum and colon tissue of cancerous patients may lead to the conclusion that selenium levels in human body are related to cancerous disease and selenium probably tends to cumulate in malignant tumour tissue.

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