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Changes of erythrocyte element status of colectomysed cancerous patients: Retrospective study☆

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ABSTRACT

Nowadays it has been established that metals and metal-induced oxidative stress act on signal transduction pathways, and are in association with cancer growth and spreading as well as in neurodegenerative disorders. In cases of several neurodegenerative diseases metals, especially Al, can be considered as a risk factor. Frequency of chemotherapy-related cognitive impairment or "chemobrain" is mentioned to be significant in literature, although very little is known about the chemotherapy-caused chemobrain and its connection with metal homeostasis alteration. Dysregulation of metal homeostasis can be assumed as one of the key factors in the progression of neurodegeneration. Therefore we were interested in studying metal element status of 27 adult patients in 3 years after their colectomy, 22 outpatients and 10 healthy volunteers in both genders. Tumour markers, laboratory parameters and metal element concentrations were determined. We found significant difference among the Al concentrations in operated patients compared with controls. Redox active Fe and Cu levels were also elevated slightly in this patient group. P and S concentrations changed in different ways, and Ca levels were slightly lower, than in healthy controls. Because of all above mentioned, examination of metal homeostasis in cancerous patients is necessary to moderate the risk of chemobrain and other redox-related disorders.

1. Introduction

Numerous surgical, radiotherapic and chemotherapic treatments as well as supportive therapies serve the reasonable quality of life, the survival and rehabilitation of cancer patients [1,2,3].

In our earlier clinical studies, we found significant differences in metal and redox homeostasis as well as in transmethylating ability in operated and treated colorectal and prostate cancer patients compared with healthy controls [4,5,6]. In different cancers the concentrations of Ca, Mg and transition metal elements were notably altered [7]. Different Ca/Mg ratios in erythrocytes were calculated in colorectal cancer patients and therefore this observation may connect to the higher osteoporosis frequency of female patients especially in cancerous processes [4]. The concentration of erythrocyte Ca of the prostatic cancer patient group, with high prostate-specific antigen tumor marker (PSA) values, was markedly lower compared to those of the healthy control group [6]. Other authors find elevated serum Ca concentration in patients with prostate cancer [8]. These observations support that in the case of prostate cancer, Ca is mobilized from the cells and the depletion of Ca is started [6].

In a "short-term" animal experiment, when rats were treated with 5 mg/kg b.w. cisplatin once on the first day and fed with CV247 (mixture of Mn gluconate, Cu gluconate, Na salicylate and ascorbic acid) for 14 days, cisplatin elevated the reactions of free radicals in the body. The main question was whether the side effects of cisplatin could be lessened with the CV247 mixture at the same time the effect of chemotherapeutic agent was evaluated as well. The treatment increased Pt, but lessened Fe, Cu, Mn, Mo and Zn concentrations in the kidney, while the treatment increased plasma Fe and Cu concentrations [9]. According to the study mentioned above, relevant alterations were found for Al and Pb [10].

Since transition metal elements are essential and play a key role in redox balance, alterations can be very important in the point of view of cancerous processes. Enormous trace metal element concentrations, such as Fe, Cu, Mn directly and Zn indirectly may catalyse the production of reactive oxygen radicals, at the

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same time these elements influence the activity of the antioxidant enzyme system [9].

One of the most feared side-effect of cancer therapy is the dementia-like "chemobrain". The symptoms show ill effect on the executive and intellectual functions of the cancer-treated patient, hence significantly influences life quality. This kind of cognitive disorder has been attached mostly to chemotherapy, but further studies revealed that many more influential factors are involved than the chemotherapeutic agent [11,12].

It is well known that cognitive and neurodegenerative disorders are also related to oxidative stress, like Alzheimer's, Parkinson's, Wilson's disease, Friedreich ataxia and prion-diseases [12–15]. As the redox system is in a close relation with metal ion homeostasis, disturbances in the metabolism of elements (mostly the dysregulation of Cu and/or Fe) are also confirmed in these neurodegenerative disorders [13–15]. While Zn serves also as a neuro-co-transmitter, its role has been also observed in them [16]. On the other hand, the alteration in the redox system in "chemobrain" has also been proven already, but there is a lack of further studies about the element concentrations [12].

Since cerebral accumulation of Al has been written down in patients with Alzheimer's and Parkinson's disease, it should be also highlighted, that this element may also have prooxidant property indirectly [17,18].

Therefore in this paper our aim was to carry out a retrospective element analysis to study Al and other redox-related metal accumulation in patients with colorectal cancer after colectomy. We also hypothesized the role of Al and d-field elements in chemobrain syndrome. To verify the hypothesis, we used erythrocyte samples because they can report on a longer period than serum or plasma.

2. Materials and methods

The retrospective study was made with the data of 49 Caucasian patients. Colectomysed cancerous patients $N = 27$ ($N_{female} = 13$; $N_{male} = 14$; mean age $\pm$ SD = 63.9 ± 8.0), operated within the last 3 years, were involved into the study. In this group, 22% of the patients had known hypertonia, 15% of the patients were obese and 11% had known diabetes mellitus. Patients of the control group $N = 22$ ($N_{female} = 13$; $N_{male} = 9$; mean age $\pm$ SD = 46.8 ± 13.8) were outpatients. In the group of the outpatients, 23% had known-about hypertonia, 18% of the patients had known-about diabetes mellitus and 9% were obese. In this group, 36% of the patients had nonviral liver diseases and at least a quarter of them had drunk constantly alcoholic beverages. While most of the patients arrived with gastritis or other abdominal pain, 14% of the whole group had known-about gastroesophageal reflux disease and also 14% had known-about lactose intolerancia. Exclusion criteria for outpatients were colorectal malignancy and inflammatory bowel diseases. Additionally 10 healthy volunteers from both genders were included in the study to compare the element contents in erythrocytes of both colectomysed and outpatient group ($N_{female} = 5$; $N_{male} = 5$; mean age $\pm$ SD = 55.3 ± 14.9), because only scarce data was found as reference intervals for the measured elements [19].

The study was made in accordance with ethical rights. Ethical permission was obtained from the Hungarian Medical Research Council (Permission number: TUKEB 167/1997, 15/2004) and the Institutional Review Board of Semmelweis University (Permission number: IKEB 3944/2004.).

2.1. Sample managements

Blood samples were collected to Vacutainer tubes for serum and prepared with standard routine laboratory methods. For element analysis, blood was collected to citrate containing tubes (Greiner Bio-One, Hungary or Vacutainer, USA.). Blood samples were centrifuged (10 min, 4 °C, 3000 rpm), plasma and white blood cells were removed and erythrocytes were diluted with isotonic solution. The erythrocyte samples were washed at least twice with isotonic NaCl solution. The haemoglobin content was determined with CHR Hemoglobin D reagent (Reagens Ltd., Hungary) and standardized to 1% haemoglobin.

2.1.1. Routine laboratory parameters

White blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution (RDW), platelet (PLT) were determined by haematological automat Advia 120 (Bayer).

The serum carbamid (CARB), creatinin (CREA), uric acid (UA), total protein (TP), albumin (ALB), total and direct bilirubin (T- and D-BIL), glutamic- oxaloacetic transaminase/aspartate transaminase (AST/GOT), glutamic-pyruvic transaminase/alanine transaminase (ALT/GPT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin/globulin ratio (Alb/Glob) were determined by Roche enzymatic in vitro assays, C-reactive protein (CRP) from the serum was measured with CRP/AUT-000 kit (Diagnosticum Ltd.). HbA1c was measured by Variant II HPLC from BioRad.

Three tumour markers were measured from the serum with LIA-mAT immunoluminometry (Budapest) kits and Berthold Lumat LB 9501 luminometer (Berthold GmbH, Germany): carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), alpha-fetoprotein (AFP).

2.1.2. Element analysis

Erytrocyte samples (3.0 g) were digested with 5 ml HNO_3 (65%), 1 ml HCl (37%) and 2 ml H_2O_2 (30%) on 200 °C in open system (in beakers covering watch-glasses). The blind was made under the same condition. After digestion, the samples were diluted to 10 ml with bidistilled water. For the measurements we didn't use reference material [10].

Standard solutions were Spectro multi-element standard solutions for ICP (CPAchem; Stara Zagora, Bulgaria) which were prepared as the blood samples.

Measurements were carried out with a Spectro Genesis simultaneous ICP–OES spectrometer equipped CCD Detector system (Kleve, Germany) and with axial plasma viewing.

The main technical parameters can be found in Table 1. Applied software is Smart analyser vision of Spectro smart studio (Version: 2.11.0630, SPECTRO Analytical Instruments GmbH, Kleve, Germany). The main criteria to select the element lines were the possible spectral interferences and the concentration range of the analyte. Quality control is summarized in Table 2 .

2.1.3. Statistical analysis

Statistical analysis was done using Microsoft Office Excel 2003 (Microsoft Corp., Redmond, USA) and Statistica 12 (StatSoft Inc., Tulsa, USA) software. Shapiro–Wilks test was used to verify

Table 1
ICP–OES general technical parameters.

Optical alignment	Paschen-runge
Wavelenght range	175–775 nm
Detector system	15 linear CCD detector
Resolution	0.029 nm
Generator frequency	27.12 MHz
Plasma power	1240 W
Coolant flow (Ar)	13.7 L/min
Auxiliar flow (Ar)	0.6 L/min
Nebulizer flow (Ar)	0.96 L/min