

Changes of erythrocyte element status of colectomised cancerous patients, a retrospective study

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Abstract

Introduction

Numerous surgical, radiotherapeutic and chemotherapeutic treatments as well as supportive therapies serve the quality of life, the survival and rehabilitation of cancerous patients (Boyle 2008, Millar 2012, Feng 2013).

One of the most feared side-effect is the dementia-like “chemo brain”. This kind of cognitive disorder has been attached mostly to chemotherapy, but further studies revealed much more influential factors than the chemotherapeutic agent (Hurria 2007).

It is well known cognitive and neurodegenerative disorders are also attached to oxidative stress, like Alzheimer’s disease, Parkinson’s disease, Wilson’s disease, Friedrich ataxia and prion-diseases (Ward 2014, Butterfield 2014; Singh 2010, Dusek 2014). As the redox system is in a close relation to the metal ion homeostasis, disturbances in the metabolism of elements (mostly the dysregulation of Cu and/or Fe) are also confirmed in these neurodegenerative disorders. While Zn serves as a neuro-co-transmitter also, its accumulation has been also observed in some neurodegenerative disorders (Braidy 2014).

In earlier clinical studies we found significant differences in metal and redox homeostasis as well as in red blood cell count (RBC), and in transmethylation ability in operated and treated colorectal and prostate cancerous patients compared with healthy controls (Blázovics 2009, Blázovics 2012, Nyirádi 2010). In different cancers the concentrations of Ca, Mg and transition metal elements were altered significantly (Váli 2008). Elevated Ca/Mg ratios in the erythrocytes were calculated in colorectal cancerous patients therefore this observation may connect to the higher osteoporosis frequency in female patients especially in cancerous processes (Blázovics 2009). The concentration of erythrocyte Ca in the patient group with prostate cancer and high PSA values was significantly lowered compared to healthy control group. Other authors also find elevated sera Ca concentration in patients with prostate cancer (Skinner 2009). This observation supports that in the case of prostate cancer Ca is mobilized from the cells and the depletion of Ca is started (Nyirádi 2010).

In animal experiment cisplatin elevated the free radical reactions in the body, although redox balance did not change notably. 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. (Máthé 2013) According to the study the most relevant alteration was found for Al and Pb (SZ).

Since Cu, Fe, Mn, Zn trace metal elements and S, Se, P non-metal essential elements are ubiquitous in biological system, and they play a key role in redox balance, therefore these alterations are very important in cancerous processes point of view. Enormous trace metal elements, such as Fe, Cu, Mn directly and Zn indirectly may catalyse the production of reactive oxygen radicals, at the same time these elements influence the antioxidant enzyme system (Szentmihályi 2014).

Since cerebral accumulation of Al has been written down in patients with Alzheimer's and Parkinson's disease, it should be also highlighted, that in a complex form this element may have prooxidant property (Kumar 2010; Exley 2004).

Therefore in this paper our aim was a retrospective element analysis to study the Al accumulation in patients with colorectal cancer after colectomy, hypothesized the role of Al in chemo brain syndrome.

Materials and methods

The 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB); the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH); microperoxidase and luminol was accepted from Sigma-Aldrich Co. (St. Louis; USA). Other solvents and commodities were obtained from Reanal-KER Ltd. (Budapest, Hungary).

The retrospective study was made with the data of 63 patients. Colectomysed cancerous patients N=28 (N_{male}=15; N_{female}=13; mean age \pm SD = 63.36 \pm 8.24) operated in 3 years were involved into the study. Patients of the control group N = 35 (N_{male}=16; N_{female}=19; age = 49.03 \pm 13.64) were outpatients. Exclusion criteria were malignancy and inflammatory bowel diseases. Additional 17 healthy volunteers from both genders were included in the study to evaluate the element contents of both cancerous and outpatient group.

Sample management

Blood samples were collected to Vacutainer Rapid Serum Tubes for serum and prepared with standard methods. For redox parameters and element analysis, blood was collected to citrate containing tubes. Plasma and erythrocyte samples were prepared by standard methods. Erythrocyte samples were determined with CHR Hemoglobin D reagent (Reagens Ltd., Hungary) and standardized to 1% hemoglobin.

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).

Carbamid, creatinin, uric acid, serum total protein, albumin, total and direct bilirubin, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), C-reactive protein (CRP), albumin/globulin ratio (Alb/Glob) were determined by Roche enzymatic in vitro assays. HbA1c was measured by Variant II HPLC from BioRad, the hematological parameters.

Carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), alpha-fetoprotein (AFP) tumor markers were measured with kits (LIA-mAT immunoluminometry, Budapest).

Parameters from the blood and the sera were measured by routine methods.

Redox parameters:

The free sulphhydryl-groups (SH) concentration in the plasma was determined with 5,5'-dithiobis(2-nitrobenzoic acid) at 440nm by the Ellmann-reaction, modified by Sedlak et al. (1986). Standard was made from glutation.

Reducing power from the plasma was determined by Oyaizu (1986) with the reduction of Fe III to Fe II; and measured at 700nm. The standard was made from ascorbic acid.

The hydrogen-donating (H-donating) ability in the plasma was measured by Hatano et al. (1988) with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) at 517nm. Inhibiting% was determined from the controll's absorbance.

A chemiluminometric assay was adapted to Berthold Lumat 9501 luminometer to study total scavenger capacity of plasma and erythrocyte samples. $H_2O_2/OH\cdot$ + microperoxidase + luminol system emits light in basic solution. The emission is in correlation with the with the amount of free radicals in the system, therefore it is in negative correlation with the scavenger capacity of the samples. The emitted amount of light was expressed in the logarithm on the relative light unit (log(RLU)) (Blázovics et al., 1999).

Element analysis

Erythrocyte samples were digested with 65% nitric acid then hydrogen peroxide. After digestion and evaporation samples were diluted to 10ml with bidistilled water. Inductively coupled plasma optical emission spectrometric (ICP-OES) method was applied for measuring content using a Spectro Genesis ICP-OES (Kleve, Germany) equipment. Spectro multi-element and Spectrum 3D standards were used to standardize the instrument. (Szentmihályi 2014).

Statistical analysis was made with Microsoft Office Excel 2003 (Microsoft Corp., Redmond, USA) and Statistica 11 (StatSoft Inc., Tulsa, USA) programs. Shapiro-Wilks test was used to verify the normal distribution. If data had normal distribution, Student's t-test for independent samples was used, if they had non-parametric, or F-test was significant Mann-Whitney U-test was used distribution. To study element contents an additional healthy group was also tested. If data had normal distribution and Levene-test was not significant, comparison was made with ANOVA and Fischer's LSD test, on the other hand Kruskal-Wallis ANOVA and multiple comparasions were made. Significance level was $p < 0.01$.

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Results

There were no significant difference between tumor markers, and they were in the normal range in general. In some cancerous patients the values were higher, because the taking of the applied blood was too close too their operation, but in some cases of the outpatations the data were also out of the normal range. These outpatients belonged to gastrointestinal disease groups, and several patients had liver diseases.

In the cancerous patients the interquartile range for CEA was 1.15 - 4.46 ng/ml, in non-tumorous patients it was 0.85 - 1.83 ng/ml, respectively (normal range: 0.8 - 2.5 ng/ml). For CA 19-9 interquartile range for cancerous patients was 5.10 - 26.0 U/ml (normal range < 33 U/ml); and for AFP it was 1.96 - 3.04 ng/ml (normal range: 0.5 - 5.5 ng/ml), respectively. In non-tumorous patients interquartile range was 3.6 - 7.8 at CA 19-9 and 1.76 - 4.65 ng/ml at AFP.

Routine laboratory parameters showed no significant differences, except RDW (Fig. X.), which was significantly higher in the cancerous group ($p < 0.001$); ALP (Fig. Y.) was also

markedly higher in this group ($p = 0.004$); but albumin/globulin ratio (Fig. Z.) was lower in the cancerous patients ($p < 0.001$).

At the redox measurement only slight differences could have been observed, without any significance. On the other hand it should be noticed by the mean values that free SH concentration, reducing power as well as plasma and erythrocyte scavenger capacity was better in the cancerous patients, only H-donating ability was lower in this group, compared to the control outpatients.

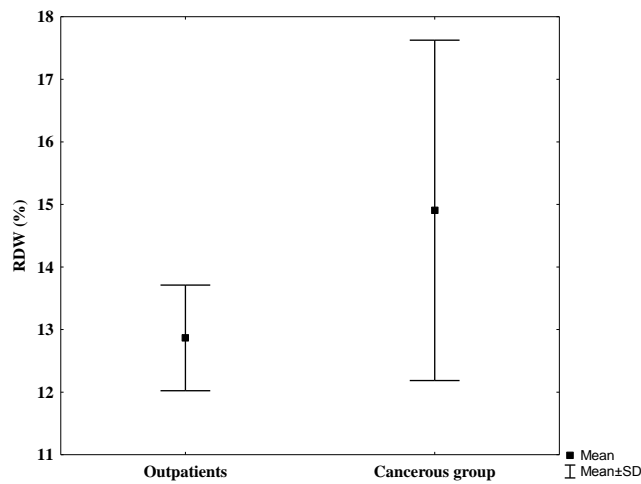


Figure X. Red blood cell distribution width (RDW) in the cancerous and control group ($p < 0.001$).

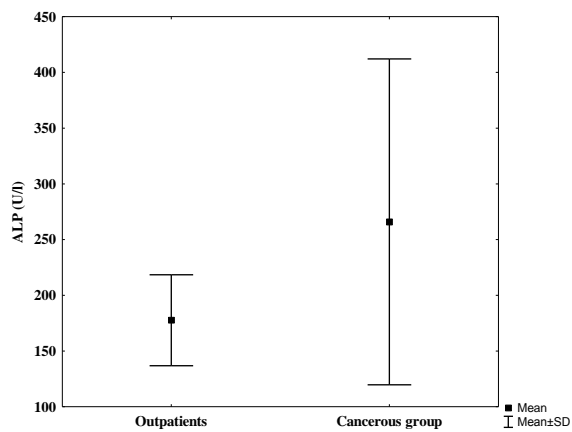


Figure Y. alkaline phosphatase (ALP) concentration in the cancerous and control group ($p = 0.004$).

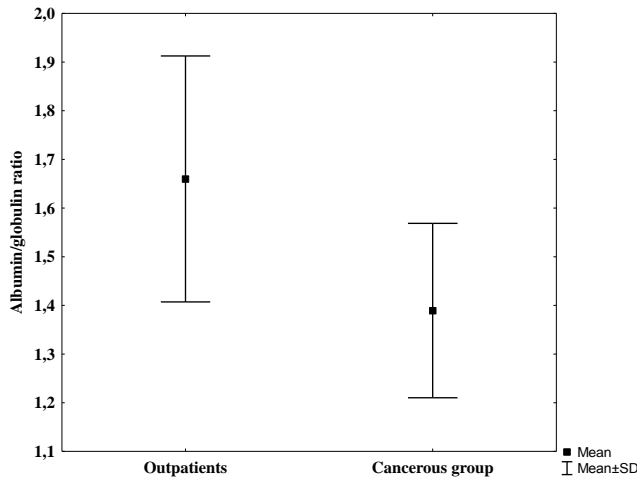


Figure Z. The albumin/globulin ratio in the cancerous and control group ($p < 0.001$).

	healthy controls (N = 17)	outpatients (N = 35)	cancerous group (N = 28)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Al (ppm)	0.413 ± 0.208	0.859 ± 1.925	1.455 ± 1.900	
Cu (ppm)	0.065 ± 0.043	0.034 ± 0.032	0.066 ± 0.078	
Mg (ppm) [*]	1.443 ± 0.401	1.624 ± 0.360	1.858 ± 0.380	p=0.002
Mn (ppm)	0.010 ± 0.007	0.012 ± 0.010	0.014 ± 0.009	
Zn (ppm) ^{***}	0.450 ± 0.138	0.376 ± 0.119	0.543 ± 0.253	p=0.001
Ca (ppm) ^{*,**}	1.650 ± 0.464	3.371 ± 2.667	3.278 ± 2.135	p<0.001
P (ppm) ^{*,***}	9.205 ± 4.198	18.074 ± 15.287	29.826 ± 12.752	p<0.001
S [†] (ppm) ^{*,**}	35.474 ± 5.698	60.799 ± 18.071	73.076 ± 28.126	p<0.001
Fe (ppm) ^{*,**}	19.498 ± 5.828	28.425 ± 5.969	32.572 ± 7.278	p<0.001

Table N

Element contents in the three different groups.

^{*} p < 0.01 between healthy controls and cancerous patients

^{**} p < 0.01 between healthy controls and outpatients

^{***} p < 0.01 between outpatients and cancerous patients

[†] N_{healthy control} = 10

The main question of this study was whether or not the element concentrations of the erythrocytes show alterations in cancer-treated patients, compared to internal medicinal patients without known tumor or irritable bowel disease in their history or healthy subjects (Table N). There were no significant differences in the Al, Cu and Mn concentrations, but the Ca ($p_{\text{Kruskal-Wallis ANOVA}} < 0.001$), the S ($p_{\text{Kruskal-Wallis ANOVA}} < 0.001$) and the Fe ($p_{\text{Kruskal-Wallis ANOVA}} < 0.001$) content were significantly lower in healthy group compared to outpatients

(healthy group/outpatients: $p_{Ca}<0.001$; $p_S<0.001$; $p_{Fe}<0.001$) and cancerous patients (healthy group/cancerous group: $p_{Ca}<0.001$; $p_S<0.001$; $p_{Fe}<0.001$) without marked differences between the outpatients and cancerous patients. In healthy patients only 10 were proper for measure the S content. Mg concentration was slightly elevated in the group of the outpatients but it was significantly higher in the cancerous group compared to healthy group ($p_{ANOVA}=0.002$; $p<0.001$). The highest concentration of Zn and P were detected in the tumorous group (Zn: $p_{Kruskal-Wallis ANOVA}=0.001$; outpatients/cancerous group: $p<0.001$; P: $p_{Kruskal-Wallis ANOVA}=0.001$; healthy group/outpatients and healthy group/cancerous group: $p<0.001$).

The highest mean values of the element contents except Ca concentration were recorded in the cancer-treated patients, however Al should be highlighted. An upper limit of Al concentration has been counted from the mean value and standard deviation of the healthy group (upper limit=mean + SD=0.621 ppm). At the healthy group only 18% of the subjects, at the outpatients 34% of the patients, but in the cancer treated group 54% of the patients had higher value than the latter maximum. The mean value of this group was 1.7 times higher than the outpatients mean value and 3.5 times higher than the mean of the healthy controls.

One tumor-treated patient had extremely high CEA (550 ng/ml) and needed further monitoring. In this case a prominently high, more than 8.0 ppm Al concentration was also detected. This patient also had higher values than the normal range in platelet number, creatinin, GGT and ALP level and lower than normal HGB, HTC, MCV, MCH, MCHC values, but gallstones were detected as well, that were associated with inadequate liver functions.

There was a patient at the non-cancerous group with extremely elevated Al concentration (10.89 ppm). In this person the pathologically high GGT value was also visible, but only uric acid level was higher then the normal value, which is in agree with the known alcoholism.

Discussion

The significantly higher RDW, which may be a biomarker of several cancer (Seretis 2013) is in consensus with Spell and coworkers (2004), and suggests that tumorous patients may have disturbed Fe homeostasis or already have anemia. The non-significant changes in the other hematologic parameters are in correlation with this literature, while chemotherapy may increases the risk of high MCV values, but in this case it does not changed significantly, and only in a few cases were higher than the normal value (Wenzel 2003; De La Cruz 2004). Deviations in hematologic parameters can be the sign of anemia, which is a common but serious case in cancers, therefore many treatments were evolved to resolve it, but only recent studies revealed, that in some cases the low methylation inhibited the synthesis of the haemoglobin (Hassan 2013; Blázovics 2008). Methyl pool is significantly lowered in cancerous patients, therefore haemoglobin biosynthesis is inhibited without free methyl group. Methyl donors are produced partly by enzymatic demethylation of some N-, S-, and O-methylated compounds. This malformation ends up in protoporphyrin-Zn incorporation to the haemoglobin, but while at prostate cancerous patients Zn depletion was found in our study excess Zn can be observed in erythrocyte samples of cancerous patients. The notably high Zn concentration in our study is in consensus with the idea of Zn-protoporphyrin incorporation to haemoglobin and supports the idea, that these patients have at least mild anemia (Blázovics 2012).

The cancer related inflammation and malnutrition (Bovio 2009) together suggest a lower albumin and higher proinflammatory protein concentration (Ionescu 2013). It should be

mentioned, that the tumor markers are also counted in the globulin concentration of the albumin/globulin ratio, while they are recently higher in cancerous patients (Azab 2013). All in all it was expected, that the albumin/globulin ratio has been considerably lower in the cancerous patients. It suggests the adverse processing in the nutrition which further influences the outcomes of the surgical treatment, but further may influence the efficacy of the whole treatment too (Ionescu 2013; Boyle 2008).

The elevated ALP activity in the blood was also expectable (Saif 2005). While the data of the patients in this study were generally in the normal range (100 – 290 U/l), Saif and coworkers suggested 160 U/l for cutoff point. It may be a better marker for liver metastasises. In this case most of our participants should be supervised more often than the regular protocols advices.

In the redox parameters, only slight differences were visible, and most of them were better in the cancerous patients. While only the H-donating activity of the plasma, was better in non-cancerous group, higher free SH level and reducing power were measured in the plasma, and lower light emission was detected with the chemiluminometric method in the plasma as well as in the erythrocytes of cancerous patients. The inducible chemiluminescence shows the level of free radicals, and is in a negative correlation with the scavenger capacity. It was unexpected, that redox parameters were as good as in the non-cancerous group, because numerous studies show, that antioxidant vitamins or other antioxidant parameters are significantly lessened in colorectal cancers (Beno 2000; Hronek 2000; Koçer and Nazıroğlu 2013; Blázovics 2008). These results support the idea of the change to a healthier life, as a result of good medical management, but on the other hand, in this study the control group had also gastrointestinal problems, and some of them may have drinking disorders.

While the concentrations of almost all elements were higher in the cancerous patients, it is not clear if metabolic element accumulation, higher level of cell death, or other mechanism can be observed. The significantly higher P level in cancerous patients compared to the other two groups maybe the sign of cell death, because hyperphosphatemia is also a signal of the rare but life threatening case, the tumor lysis syndrome (Kim 2014). The importance of P concentration is discussed in surgical cases, and in the future may become a crucial prognostic factor (Ye 2013).

Zn concentration has changed diversely in the two patient groups. Its concentration has been lowered in the outpatients, but elevated in the cancerous group, causing significant difference between the two patient groups. The elevated Zn concentration may also suggested by some already reported cases, that determined elements in the blood (Shenberg 1995) and measured increased urinary excretion (Melichar 1994; Hronek 2000). In the study of Shenberg, the increased blood Cu level in cancerous patients could be also noticed, but in their case the Fe content was lowered in the cancerous patients. Elevated Cu level was also suggested by two other studies (Beno 2000; Gupta 1993).

Only scarce data could be found about Al contents in studies about colorectal tumors, but Lavilla et al. (2009) has found elevated Al and essential element content in cancerous tissues while compared the healthy to the cancerous tissues in 38 colorectal cancerous patients. This study can confirm the elevated Al-content in our study. One cancerous patient had more than 8.0 ppm Al concentration, while the mean value was about 1.46 ppm still in the cancerous group. It was also associated with poor prognostic factors, like anemia-like symptoms, hepatic disorders, maybe a liver metastasis that the high CEA and ALP value can predict. As the highest Al concentration in the non-cancerous group was also associated with high GGT

value, it may also confirm the hepatotoxicity of Al (Shati 2010). On the other hand the known alcoholism increases GGT parameter too and the Al accumulation maybe just worsens the hepatic disorder. These results raise the question if the high Al concentration is the inducer or the result of hepatic disorders and further worsens the function of the liver.

On the other hand, it is well known, that elevated element concentrations, for instance Al; Fe; Cu; Zn level can cause disturbances in the redox homeostasis of healthy tissues, for example in neurons as it has been proven in many studies about Alzheimer's, Parkinson's or Wilson's disease, Friedrich ataxia and prion-diseases (Ward 2014, Butterfield 2014; Singh 2010, Dusek 2014; Braidy 2014). Since the chemotherapy associated cognitive impairment is not clearly understood yet, but oxidative stress has been confirmed in these cases, researches about associations with the element contents are necessary (Hurria 2007; Butterfield 2014). Taken together our study revealed the elevated source of redox related elements in colorectal cancer treated patient, in particular the the Al content, the significantly higher Zn concentration compared to outpatients, and Fe content compared to healthy subjects that may be factors in "chemo-brain" or other oxidative stress related disorders in cancer and/or chemotherapy.

Conclusion

In this retrospective study colorectal cancerous patients were compared to internal medicinal outpatients. Routine laboratory parameters were generally in the normal range and only RDW, ALP and albumin/globulin ratio differed significantly. There were no significant differences in the redox parameters too. The main question was whether the cancerous state differ the element contents of the erythrocytes, therefore an additional healthy group was added. The measured elements (Al, Cu, Mg, Mn, Zn, Ca, P, S and Fe) were in a higher concentration in cancerous patients compared to healthy subjects. All measured elements, except Ca were similarly in a higher concentration in cancerous patients compared to outpatients. All in all an element accumulation can be observed in the cancerous state. The higher amount of Al, Cu, Zn and Fe should be highlighted, as the dysregulation of these elements are well known features in neurological disorders, like Alzheimer's, Parkinson's or Wilson's disease, Friedrich ataxia and prion-diseases. This result suggests, that "chemobrain" and other redox system related disorders in cancerous state and/or under chemotherapy may be related to disturbed element homeostasis as well.

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