

Copper-Zinc ratio and nutritional status in colorectal cancer patients during the perioperative period¹

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ABSTRACT

PURPOSE: This study aimed to determine Cu/Zn ratio, nutritional and inflammatory status in patients during the perioperative period for colorectal cancer.

METHODS: The study included patients with histological diagnosis of colorectal adenocarcinoma (Cancer Group, n=46) and healthy volunteers (Control Group, n=28). We determined habitual food intake, body composition, laboratory data of nutritional status, serum calprotectin and plasma Cu and Zn concentrations. Mann-Whitney U-test was performed between-group comparisons and Spearman correlation test for correlations between the variables.

RESULTS: Individuals in the Cancer Group presented significantly lower BMI, fat mass, plasma hemoglobin, total protein and albumin as compared with the Control Group. Serum calprotectin [70.1 ng/mL (CI95% 55.8-84.5) vs. 53.3 ng/mL (40.3-66.4), p=0.05], plasma Cu concentrations [120 µg/dL (CI95% 114-126) vs. 106 µg/dL (CI95% 98-114), p<0.01] and the Cu/Zn ratio [1.59 (CI95% 1.48-1.71) vs. 1.35 (CI95% 1.23-1.46), p=0.01] were higher in patients with colorectal cancer than in controls. Additionally, the Cancer Group showed negative correlations between the Cu/Zn ratio and Zn intake, hemoglobin, serum albumin, and positive correlation between the Cu/Zn ratio and serum calprotectin.

CONCLUSION: These results indicate that an increased plasma Cu/Zn ratio and serum calprotectin, and decreased protein values may be a result of the systemic inflammatory response to the tumor process.

Key words: Zinc. Cooper. Nutritional Status. Inflammation. Colorectal Cancer.

Introduction

The trace elements zinc (Zn) and copper (Cu) are essential for human health. Dietary factors, including a wide range of phytate intakes, cellular location, cell-specific expression, and regulation of the transporters involved in enteric Zn absorption are important for bioavailability this mineral¹. Approximately 70% of circulating Zn is bound to serum albumin². Zn is a constituent of more than 300 enzymes involved in a broad spectrum of activities, including anti-inflammatory and antioxidant effects³ and genetic transcription regulation⁴. Dietary Cu is absorbed mainly in the duodenum and is chelated by metallothioneins in the cytoplasm of cells⁵. Cu binds to proteins involved in a variety of biological processes⁶ such as the formation, maturation and stabilization of connective tissues and structure of the extracellular matrix⁷. Cu is involved in the detoxification of reactive oxygen species as it binds to metallothioneins under reducing conditions and influences the activity of antioxidant enzymes⁶. Cu is also essential for the immune response, including the production of IL-2 by activated lymphocytic cells⁷.

The Cu/Zn ratio has been correlated with nutritional status in institutionalized elderly⁸ and patients under peritoneal dialysis⁹. Also, this ratio is considered as a marker for asthma severity¹⁰ and prostate cancer detection¹¹. In the elderly, increased Cu/Zn ratio was a predictive factor of mortality and it was associated with reduced serum albumin and increased inflammatory markers, like C-reactive protein and erythrocytes sedimentation rate¹².

Calprotectin (S100A8/A9) accounts for more than 60% of total soluble proteins in the cytoplasm of neutrophils¹³. Serum calprotectin has been suggested as a biomarker of chronic inflammation¹⁴, and to be correlated with acute phase response markers' concentrations¹⁵. The release of calprotectin by activated leukocytes¹³ precedes the increase in liver protein synthesis in response to inflammatory stress^{13,15}. In view of the detection of early acute inflammatory changes, the simultaneous determination of calprotectin and hepatic markers levels would be clinically useful for monitoring several inflammatory conditions¹⁵.

Colorectal cancer is one of the major causes of death by cancer in the western world, and surgery for excision of the primary tumor is undertaken in 80% of patients¹⁶. Preoperative colorectal cancer patients are not usually considered to be at risk of malnutrition¹⁶. Although these patients may be classified as overweight or obese according to the BMI, some of them have a weight loss greater than 10%, indicating a certain degree of malnutrition¹⁶. So far, to our knowledge, there is no available study correlating nutritional status and inflammatory status with Cu/Zn

ratio in colorectal cancer. The present study aimed to compare and correlate the plasma Cu/Zn ratio with nutritional and inflammatory status in colorectal cancer patients during the perioperative period.

Methods

The study was approved by the local Research Ethics Committee (Proc#14102/2010) and data were collected between May 2011 and December 2012 in a Brazilian university hospital. Informed consent was obtained from all individual participants included in the study. A convenience sample of 46 patients (22 male), aged 65.8 years (62.0-69.7) in the perioperative period for stage II, III or IV adenocarcinoma of colon or medium/high rectum (Cancer Group) participated in the study. All patients had no previous chemotherapy or radiotherapy and were free of the recurrent neoplastic lesion. A group of 28 healthy volunteers (14 male), aged 61.4 years (58.1-64.8) (Control Group) was also included in the study. These volunteers were recruited by a community program, and use of a local press and radio. Exclusion criteria included the history of neoplasms, autoimmune diseases, chronic inflammatory diseases, active infection, liver or kidney diseases, and use of supplements containing Zn and Cu.

Assessment of food intake and body composition

At the beginning of the study, a semi-quantitative food frequency questionnaire (SQFFQ) was applied to evaluate subjects' habitual food intake in the preceding six months. Daily intake of nutrients was determined by using the software (NutWin, version 2.5a, UNIFESP, São Paulo, Brazil).

For anthropometry, height (m) and body weight (kg) were measured using standardized techniques, and the Body Mass Index (BMI=[weight/height², kg/m²]) was calculated. Body composition was determined by bioelectrical impedance analysis (Biodynamics BIA 450 Analyzer, Biodynamics Corporation, Shoreline, WA, USA).

Biochemical analyses

Routine laboratory analyses included serum levels of total protein and albumin (colorimetry), determined with an automatic biochemical analyzer (Konelab 6.0, Winer, Argentina). Hemoglobin levels were determined by photometry, using the ABX Pentra DX120 (Horiba Ltd, Kyoto, Japan). Serum calprotectin (S100A8/A9, MRP8/14) was measured by ELISA (Multiskan FC Microplate Photometer, Thermo Scientific Uniscience) using

a commercially available kit (DRG Diagnostics, EIA5111; Springfield, New Jersey, USA). The standard curve ranged from 3.9 to 250 ng/mL. Plasma zinc and copper values were determined by flame atomic absorption spectrophotometry (Varian, model AA200, Melbourne, Australia), at a wavelength of 213.9nm and 327.4nm, respectively. The normal values for plasma zinc and copper were set at 70-140µg/dL and 50-120µg/dL, respectively.

Statistical analysis

Mann-Whitney U-test was used for comparisons between demographic and clinical features of groups, and the results are shown as geometric mean and 95% confidence interval. Spearman's correlation coefficient was used for correlation analyses between variables and the Cu/Zn ratio. Data analyses were performed with

Statistica software (version 8.0; StatSoft Inc., Tulsa, OK, USA). The level of significance was set at 5%.

Results

Food intake, body composition and laboratory data are shown in Table 1. Energy, protein and zinc intake was similar in the Cancer Group as compared with controls in the six months prior to the study. Individuals of the Cancer Group exhibited lower body weight, BMI and fat mass as compared with the Control Group. Colorectal cancer patients presented lower hemoglobin, total protein and albumin serum concentrations than controls. Plasma Cu concentrations were higher in patients with colorectal cancer than in controls, and plasma Zn was similar between groups. Hence, a higher Cu/Zn ratio was found in the Cancer Group than in the Control Group.

TABLE 1 - Mean and IC 95% of food intake, body composition and laboratory data of patients with colorectal cancer (Cancer Group) in the perioperative period and healthy volunteers (Control Group).

	Cancer Group (n=46)	Control Group (n=28)	P-values
Habitual food intake			
Energy (kcal)	1962 (1774-2150)	2113 (1811-2416)	0.36
Protein (g)	67.7 (59.1-76.4)	82.9 (65.8-100.1)	0.15
Carbohydrate (g)	253 (222-284)	283 (235-331)	0.21
Lipids (g)	77.9 (70.0-85.8)	75.8 (65.0-86.6)	0.88
Zinc (mg)	8.3 (7.3-9.3)	9.7 (7.7-11.7)	0.29
Copper (mg)	1.24 (1.03-1.46)	1.42 (1.16-1.68)	0.09
Bodycomposition			
Weight (kg)	66.9 (62.4-71.3)	73.5 (67.8-79.2)	0.04
BMI (kg/m ²)	25.2 (23.8-26.6)	28.2 (26.6-29.7)	0.01
Lean mass (kg)	46.4 (43.2-49.7)	48.1 (43.3-52.9)	0.59
Fat mass (kg)	19.8 (17.2-22.3)	25.4 (22.3-28.5)	<0.01
Laboratory data			
Hemoglobin (g/dL)	11.4 (10.8-11.9)	14.3 (13.7-14.9)	<0.01
Total protein (g/dL)	6.3 (6.1-6.5)	6.9 (6.7-7.1)	<0.01
Albumin (g/dL)	3.8 (3.6-3.9)	4.2 (4.2-4.3)	<0.01
Calprotectin (ng/mL)	70.1 (55.8-84.5)	53.3 (40.3-66.4)	0.05
Copper (µg/dL)	120 (114-126)	106 (98-114)	<0.01
Zinc (µg/dL)	77.7 (73.3-82.1)	80.0 (75.9-84.1)	0.50
Copper/Zinc	1.59 (1.48-1.71)	1.35 (1.23-1.46)	0.01
Body mass index: BMI			

In the Cancer Group there was a weak negative correlation between Cu/Zn ratio and Zn intake ($r=-0.24$), serum albumin ($r=-0.37$) and hemoglobin ($r=-0.42$), and a weak positive correlation between Cu/Zn ratio and serum calprotectin ($r=0.28$).

Discussion

This study has found that patients with colorectal cancer presented lower body weight, BMI and fat mass compared

to healthy volunteers. Besides, the Cancer Group had lower hemoglobin, total protein and albumin serum levels, and higher serum calprotectin, plasma Cu and Cu/Zn ratio than controls.

The assessment of habitual food intake by the SQFFQ revealed that the nutrient intake in patients with colorectal cancer was similar than in healthy controls, including Cu and Zn, met the Dietary Reference Intakes (DRI). This result is different from the study reported by Ravasco et al. (2004)¹⁷, which showed that patients in the perioperative period of head and neck, esophagus, stomach and colorectal tumor surgery had low nutrient intake as assessed by the 24-hour recall method. The discrepancy between results may be explained by differences in the methods used to estimate food consumption in these studies, although some studies show correlations between nutrient intakes measured by a frequency questionnaire and multiple 24-hour dietary recalls or diet records¹⁸.

Anthropometric data of patients were similar to those found in the literature¹⁹. The prevalence of nutritional disturbances in colorectal cancer patients depends on the definitions of nutritional risk and malnutrition adopted at the time of evaluation^{19,20}. Some studies have demonstrated a high prevalence of overweight and obesity in these patients¹⁹, indicating little effect on nutritional status, differently from other advanced neoplasms in the gastrointestinal tract²¹. However, it is worth mentioning that BMI may continue within normal range even after weight loss. History of weight loss was evaluated by the Subjective Global Assessment (SGA), which showed that 35% of patients with colorectal cancer were moderately or severely malnourished or at nutritional risk²⁰. On the other hand, the Malnutrition Screening Tool (MUST) and the Nutritional Risk Index (NRI) identified undernutrition in 44% and 53% of colorectal cancer patients, respectively²⁰.

In colorectal cancer, food intake is slightly affected with no digestive or absorptive disturbances, and intestinal obstruction occurs only in advanced cases of the disease. On the other hand, inflammatory pathways are constitutively active in most cancers²¹ and may lead to reductions in body protein levels, regardless of food intake. Although colorectal tumors trigger inflammatory stress responses, albumin levels are generally within normal range or slightly reduced²². In this context, increased calprotectin levels in our cancer patients indicate its possible role in inflammation-associated carcinogenesis¹⁴. This increase has been related to the persistent innate immune cell activation in many epithelial cancers¹⁴ and detection of calprotectin-positive myeloid cells within the tumor stroma in colorectal cancer²³.

Also, our findings regarding Cu plasma concentrations are in accordance with those observed in uterine cervical²⁴ and gut cancer²⁵, reporting higher levels of Cu as compared with healthy

volunteers. Incremental changes of inflammatory biomarkers have been associated with augmented plasma Cu²⁶. Approximately 90-95% of the total circulating Cu are bound to ceruloplasmin⁷, whose concentrations are elevated in response to inflammation, infection and various chronic diseases^{6,7}. During stress and trauma conditions, the proinflammatory cytokines IL-1 and IL-6 stimulate the synthesis of acute-phase reactive protein by the liver⁷, which may explain the increase in plasma Cu levels. Zn deficiency was not observed in our patients, different from previous studies that reported low Zn levels in uterine cervical²⁴ and gut cancer²⁵ patients.

A greater Cu/Zn ratio occurred in our Cancer Group than in the Control Group, similarly to previous studies on uterine cervical cancer²⁴. Tuberculosis patients had a high level of copper and copper/zinc ratio when compared to healthy controls, and the alteration was more pronounced in those coinfecting with HIV²⁷. The Cu/Zn ratio has been pointed out as a diagnosis and prognosis factor of inflammation in lymphoma and leukemia²⁸, gastric²⁹ and breast cancer³⁰.

A limitation of this study was the relatively small numbers of patients and controls, although the Cancer Group was homogeneous regarding tumor site and histological diagnosis. Despite small sample size, we found negative correlations between the Cu/Zn ratio and the nutritional parameters in patients with cancer. In subjects under peritoneal dialysis, elevated Cu/Zn levels were associated with undernutrition, increased oxidative stress, inflammation and deterioration of immune function⁹. The Cu/Zn ratio increased proportionally with increasing levels of prostate specific antigen in patients with prostate cancer¹¹, and correlated with lipid peroxidation markers in asthma patients¹⁰.

Conclusion

This study has shown that the Cu/Zn ratio and the inflammatory marker calprotectin are increased, and protein levels decrease in patients with colorectal cancer. These findings suggest that the Cu/Zn ratio may be an inflammatory-nutritional biomarker, resulting from the inflammatory response to the tumor process. Further work might investigate whether other immunological and inflammatory markers could affect trace element status and clinical progression in cancer patients.

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