Journal of King Saud University – Science (2011) 23, 337–340

King Saud University
Journal of King Saud University – Science
www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Distribution of trace elements like calcium, copper, iron and zinc in serum samples of colon cancer – A case control study

Nora A. Al Faris [a], Dilshad Ahmad [b,*]

[a] Princess Nora bint Abdulrahman University, P.O. Box 84428, Riyadh, Saudi Arabia
[b] College of Food and Agricultural Science, Department of Food and Nutrition, P.O. Box 2460, King Saud University, Riyadh, Saudi Arabia

Received 22 June 2010; accepted 12 July 2010
Available online 15 July 2010

KEYWORDS
Colon cancer;
Trace elements;
Calcium;
Copper;
Iron;
Zinc

Abstract The pathogenesis of a number of diseases like cardiovascular diseases, diabetes and cancer has been associated with changes in the balance of certain trace elements. In the present study we aimed to investigate the levels of trace elements like calcium, copper, iron and zinc in colon cancer patients in comparison with healthy controls. Serum samples were collected from 256 colon cancer patients and 180 healthy age and sex matched controls. Trace element levels were detected using commercially available kits and an Auto-Analyzer (ChemWell 2910, Awareness Technology, and USA). The concentrations of calcium, copper and iron were not significantly different in patients in comparison with healthy controls. The concentration of zinc was significantly lower in colon cancer patients ($p = 0.001$) as compared to normal subjects. Deficiency of zinc may play a role in the development of colon cancer or may contribute to damage already underway. Zn may represent an independent risk factor for colon cancer and therefore a possible target for prevention.

1. Introduction

Although, the risk factors do not fully explain variations in the incidence of complex disorders generally in cancer and colon cancer in particular. Epidemiological studies have implicated perturbations in the trace elements in the etiology of a number of diseases including cancer. Trace elements are essential for numerous metabolic and physiological processes in the body (Merz, 1981). Therefore, imbalances in the optimum levels of trace elements may affect biological processes and have been associated with many diseases including heart autoimmune, cancer, renal failure and neurological disorders (Dar et al., 2008; Fuster et al., 1992; Shokrzadeh et al., 2009). Most of

* Corresponding author. Tel.: +966 507990480.
E-mail addresses: nawers1@hotmail.com (N.A. Al Faris), drdilshadahmad@gmail.com (D. Ahmad).

1018-3647

Peer review under responsibility of King Saud University.
doi:10.1016/j.jksus.2010.07.017

Production and hosting by Elsevier

these diseases have been associated with changes in the balance of trace elements like magnesium (Mg), copper (Cu), zinc (Zn), calcium (Ca), iron (Fe) etc. Mn, Zn, Fe and Cu accomplish decisive functions in maintaining human health and play an important role in the synthesis and structural stabilization of both proteins and nucleic acids. These elements are incorporated into the enzymes and complex carbohydrates e.g., Cu is a cofactor for numerous enzymes and plays an important role in development (Desai and Kaler, 2008). Zinc is important for the functioning of more than 300 enzymes from all six enzyme classes and some of them are related with DNA and RNA synthesis (Vallee et al., 1991; Tudor et al., 2005; Vallee and Falchuk, 1993). It has a vital role in immune system and is essential for the optimal function of a variety of physiological and biochemical processes (Tudor et al., 2005). Based on the bioinformatics estimates it has been reported that 10% of the proteome contain Zn binding motives (Andreini et al., 2006). Iron, apart from its presence in all body cells, is the most abundant transition metal found in several tissues. It is involved in many processes including DNA, RNA and protein synthesis. Low serum iron has been reported in a variety of motor disorders (Peralta et al., 1994). Copper is an important trace metal which plays a fundamental role in the biochemistry of the human system. It is an important component of several metalloenzymes including tyrosine and dopamine hydroxylase (Livesay, 2003). Copper has been suggested to play an important role in several disorders (Wallwork, 1987). Calcium apart from being vital for healthy teeth and bones plays a crucial role in the functioning of nerves and muscle tissue.

A number of studies carried have shown that dietary calcium intake is associated with a reduced incidence of colon cancer among middle-aged subjects. These observations suggest that alterations in essential trace elements like Cu, Fe, Zn and Ca may play an important role in the pathogenesis of complex diseases including colon cancer. Very few reports are available on the concentration of these trace elements in colon cancer patients. This work was under taken to estimate the levels of trace elements like Ca, Cu, Fe and Zn in serum of colon cancer patients in comparison with healthy controls. The analyses of these trace elements may have a prognostic significance for colon cancer.

2. Subjects and methods

2.1. Study subjects

The study consisted of 256 colon cancer patients (males: females = 176:80) from University Hospital. Informed consent was obtained from all the subjects included in the study. The study was approved by the ethical Committee of the study hospital. Each patient was examined by a qualified oncologist to confirm the diagnosis. Patients were differentiated by computed tomography scans and magnetic resonance imaging. One hundred and eighty healthy individuals matched for sex and age formed control group (males:females = 124:56). They were recruited simultaneously from the same demographic area. Information on demographic characteristics and risk factors were collected by using a structural questionnaire. Risk factors included hypertension, diabetes, and smoking. According to joint National Committee VI-VII, hypertension was defined as a systolic blood pressure >140 mm Hg and/or a diastolic blood pressure >90 mm Hg based on the average of the two blood pressure measurements, or a patient's self-reported history of hypertension or antihypertensive use, supported by documents (Britoy and Bystroya, 2003). Diabetes was diagnosed if fasting plasma glucose was >110 mg/100 ml or patient was on ant diabetic medication (Radhakrishnamurthy, 2003). Smokers were defined as those reporting daily smoking. Ex-smokers and occasional smokers were classified as non-smokers (Glader et al., 2000).

3. Collection of blood samples and trace element analysis

Venous blood (5 ml) was collected from patients as well as controls after overnight fasting, into a metal free plastic tube and allowed to clot at room temperature for 1 h. Then the blood sample was centrifuged at 3000 rpm for 15 min at room temperature to separate the serum. The serum was aliquoted into micro centrifuge tubes and stored at −80 °C until analysis. Zn and Fe were analyzed using kits obtained from Centronic Gmbh, Germany and Ca and Cu were analyzed by kits from Chema Diagnostisa, Italy. The sample pretreatment was carried out according to the instructions of the kit manufacturer.

The measurement principle for the different trace elements is as follows:

Calcium: Arsenazo (III) combines with calcium at slight acidic pH to form a blue complex, the absorbance of which is measured at 660 nm. The reaction has high specificity and interference from Mg is avoided due to pH (Leary et al., 1992).

Copper: 3,5-Di-Br-PAESA combines with Cu (II) to form a blue–violet complex, the absorbance is measured at 580 nm. The reaction has high specificity and interference from other cations is avoided, due to specific pH and environment (Abe et al., 1989).

Iron: Fe ions are dissociated from its carrier protein, transferring in an acid medium and simultaneously reduced to ferrous state. The ferrous ions react with chromogen nitro-PAPS to a color-complex highly specific. The absorbance is measured at 578 nm and is directly proportional to the iron content (Makino et al., 1988).

Zinc: Zn forms a red chelate complex with 2-(5-bromopyridylazo)-5-(npropyl-*N*-sulphopropylamino)-phenol. The absorbance is measured at 560 nm and is proportional to the concentration of total zinc in the sample (Johansen and Eliassonn, 1987).

3.1. Statistical analysis

The results were expressed as mean ± SD by using Sigma stat (Windows Version 11.5) software. The analytical data was subjected to one way ANOVA using the same software. The significance was set at $P < 0.05$.

4. Results

During the study period, 256 colon cancer patients and 180 controls were included in the study. The demographic data of the patients and controls has been presented in Table 1. Mean age was 48 years in the former group and 47 years in the latter. Hypertension was the most common risk factor followed by diabetes, and smoking. Risk factor profile of colon cancer patients revealed hypertension in 71%, diabetes in 43%, smoking in 45% and family history of colon cancer in

Table 1 Demographic features of colon cancer patients and controls.

	Patients (n = 256) (%)	Controls (n = 180) (%)
Age (mean ± SD)	48.53 (16.34)	47.01 (17.78)
Male:female	176:80	124:56
Hypertension	71	50
Diabetes	43	31
Smokers	45	40
Family history of colon cancer	25	12

Table 2 Trace element levels in colon cancer patients and controls.

	Colon cancer patients mean ± SD	Healthy control mean ± SD	One way ANOVA
Calcium level (μg/dl)	13.10 ± 8.86	9.81 ± 6.26	P = 0.070
Iron level (μg/dl)	33.23 ± 47.60	40.71 ± 67.94	P = 0.544
Copper level (μg/dl)	154.60 ± 91.71	152.08 ± 112.56	P = 0.760
Zinc level (μg/dl)	65.39 ± 61.01	117.14 ± 63.27	$P \leqslant 0.001$

25% subjects. In the control group 50% had hypertension, 30% were diabetic, 40% smokers and 12% had family history of colon cancer. Serum trace elements concentrations are presented in Table 2. The mean Ca level of the colon cancer patients was 13.10 μg/dl, which was more than the mean Ca level of the healthy controls (9.81 μg/dl) but did not reach statistical significance. Fe and Cu levels of colon cancer patients were not significantly different in comparison with the healthy volunteers ($P > 0.05$). Only serum concentration of Zn was decreased significantly ($P < 0.001$) in colon cancer patients compared to that of controls. The mean Zn level in patients was 65.39 μg/dl and in controls it was117.14 μg/dl.

5. Discussion

The present work was designed to determine the level of trace elements in colon cancer patients and compare these with healthy controls. Changes in trace elements have been examined in many diseases like cardiovascular diseases, Diabetes, neurological disorders and cancers (Shokrzadeh et al., 2009; Desai and Kaler, 2009; Waggoner et al., 1999). Changes in trace element levels may have prognostic significance in complex disorders including colon cancer. A number of molecular roles for the trace elements like Fe and Zn have been identified in differentiation and apoptosis (Emel et al., 2005). Similarly Ca has been shown to play a primary role in the development of cell injury in different pathological states of vital organs. Disturbances of calcium homeostasis may be induced in three different sub cellular compartments including cytoplasm, mitochondria or the endoplasmic reticulum (Wulf, 2000). According to the traditional calcium hypothesis the cell injury is induced by a marked increase in cytoplasmic calcium activity during stress. In fact injury has been shown to be associated with both increase and decrease in cytoplasmic calcium activity (Cuajungco and Lees, 1997). This shows that any significant modification of trace element status might lead to the disease development. The basis for the pathology induced by these trace elements is under investigation. Several mechanisms have been proposed. Some investigators proposed that although these substances may not initiate damage, changes in their levels may contribute to damage already underway (Marniemi et al., 2005).

In the present study the levels of Ca, Fe and Cu were not significantly different in colon cancer patients in comparison with healthy controls. Although Ca levels seemed to be high in colon cancer patients, it did not reach statistical significance. Patients also tended to show low iron concentrations in comparison with healthy controls although devoid of statistical significance. Marniemi et al. (2005) have reported that low serum Fe concentrations proved indicative of increased risk for both acute myocardial infarction (Arnaud et al., 1994). Serum Zn levels were significantly reduced in colon cancer patients ($P < 0.001$). The serum Zn levels have been reported to decrease within first three days after acute myocardial infarction (Katayama et al., 1990). Further, Katayama et al. (1990) have reported that prognosis seemed to be much worse in those patients whose serum Zn concentrations were remarkably decreased with 12 h from admission (Chandra et al., 1994). Zn has a key role to play in the several body functions because it participates in the superoxide dismutase and Zn-thionine enzymes to reduce oxidative stress (Forte et al., 2005).

The decreased levels in serum Zn in colon cancer patients may be due to the mobilization of circulating Zn to the colon cancer tissue and its involvement in the antioxidant defense since the patients might be under higher oxidative stress. Zn is an essential cofactor in a variety of enzymes and has antioxidant-like properties. Therefore, it can stabilize macromolecules against radical induced oxidation in vitro as well as limit excess radical production (Dhalla et al., 1999). A lower level of zinc can also aid in the deterioration of cancer affected colon tissue causing additional damage. In our colon cancer patients also there was a trend for higher copper levels. However, it was not significantly different in comparison with the healthy volunteers. Zn levels have also been shown to be significantly low in some cancers (Dar et al., 2008). Therefore, trace element deficiency or excess may be implicated in the development or progression of colon cancer. The present study point towards a role of trace element imbalance, especially Zn in the colon cancer. However, is it a direct cause–effect relationship or a consequence remains a big question? Further studies are warranted to determine whether change in serum Zn may represent an independent risk factor for the development of colon cancer and in turn a possible target for preventive intervention.

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