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Research Article

Investigated of Ceruloplasmin Activity and Related Elements, Copper and Zinc in Patients with Colon Cancer

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Abstract

Colon cancer is still one of the leading causes of cancer death worldwide and the second most common cause of mortality from cancer. It is linked to numerous reasons such as socioeconomic status, the dramatic change in dietary habits, the presence of refrigeration, chemical preservatives, and the environmental changes. Our study involved the estimation of Ceruloplasmin activity (Cp), copper and zinc levels in patient with colon cancer. Serum samples were collected from 50 patient with colon cancer from Al-Hussain hospital / Kerbala , digestive and liver disease education hospital medical city in Bagdad/ Iraq and teaching oncology hospital Bagdad medical city/Iraq .The results showed decrease in the Cp activity but not significant in patients with colon cancer comparing with the control, whereas Cu concentration was significant increase ($p< 0.0001$) in patients so there was significant decrease ($p< 0.0001$) in Zn concentration in patients with colon cancer compared with the control group. Also the patients were divided into four groups according to the stage of cancer, the results revealed no any significant differences ($p > 0.05$) between the stages in the concentrations of parameters under study except there was significant differences ($p<0.05$) in concentration of copper when comparing stage 3 versus stage 4. The aim of this study is to find the relation between ceruloplasmin and related elements in patients with colon cancer.

Keywords*: colon cancer, ceruloplasmin, copper, zinc.*

1. Introduction

Colon cancer is a neoplastic disease of the large intestine, which can be derived from both inherited and somatic genetic alterations that develop over the course of a lifetime. Prevention together with early detection and treatment are the keys to a successful outcome for this very common disease (Yeatman, 2001).Colon cancer is still one of the leading causes of cancer death worldwide (Waldner, 2006) and the second most common cause of mortality from cancer (Cappell, 2005). It is linked to numerous reasons such as socioeconomic status, the dramatic change in dietary habits, the presence of refrigeration and chemical preservatives, in addition to the environmental changes. The age incidence was found to be younger, specifically female patients than that was reported in other countries (Al-Humadi, 2008).

Ceruloplasmin is a glycoprotein with a polypeptide chain including 1046 amino acid residues. This protein belongs to the group of acute phase reactants and is a principal copper containing protein of plasma. It is therefore a major copper carrier in plasma (Philippe, *et al*, 1988). It is an effective antioxidant and is one of the acute phase reactants, whose concentration in plasma rises after tissue injury. Most acute phase proteins can be thought of as protecting the organism as a whole, from the possible ill effects caused by the release of free radical oxidation products. This suggests that the organism might respond by raising the antioxidant efficiency of plasma, by elevated ceruloplasmin levels (Winyard, *et al*, 1989).

Zinc is one of the most essential metals in organisms, and zinc-binding proteins play an important role in a variety of biological processes such as transcription regulation, cell metabolism and apoptosis (Banas, *et al.*, 2011). Ceruloplasmin does not bind to Zn ions in plasma, Zn binds to a many proteins such as albumin, α2-macroglobulin, transferrin and histidine rich glycoprotein (Borth, 1992). Zinc plays an important role in the functions of transcription factor, antioxidant defense system and DNA repair. Dietary deficiencies in the intake of zinc can contribute to single and double-strand DNA breaks and oxidative modifications to DNA that increase risk for cancer development (Ho, 2004) It is also involved in a variety of general cellular functions such as cell signal transduction, transcription and replication. Zn has important role in prevention of cancer formation by stabilizing the structure of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and ribosomes. Copper is a redox-active transition metal that can participate in single electron reaction and catalyze formation of free radicals. It is also an essential element for formation of hemoglobulin, myelin, collagen, and melanin. It has been suggested to

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play an important role in several disorders and normal immune function (Emre, *et al.*, 2013). It has a role in many enzymes involved in oxidation ceruloplasmin (Johannesson, *et al.*, 2012)

Materials and methods

Subjects

Blood samples were collected from 50 patient with colon cancer and 33 healthy with no history for any gastro-intestinal problem, in period from October 2013 to March 2014 from(Al-Hussain Hospital /Kerbala, Digestive and Liver Disease Education Hospital Medical City/ Bagdad and Teaching Oncology Hospital /Bagdad Medical City/ Bagdad /Iraq),their aged between (20-70) year, 21 female and 29 male. All patients were subjected to a personal interview using especially designed questionnaire format full history with detailed information (age, sex, symptoms, BMI, family history of cancer and other disease.

Samples Collection and preparation

Five milliliters of venous blood were drawn, the blood allowed to clot in room temperature and centrifuged at 3000 rpm for 15 minutes, and serum were separated and stored at -20℃ until analysis.

Determination of Ceruloplasmin oxidase activity

The enzymatic assay of ceruloplasmin activity was accomplished using Schosinsky *et al*., (1974) method who based on measured of serum ceruloplasmin from its oxidase activity by using o-dianisidine dihydrochloride as substrate.

Determination of zinc (Zn)

Zinc was determined by spectrophotometric method by using a spectrum kit.

Determination of copper (Cu)

Copper was determined by spectrophotometric method by using a spectrum kit

Statistical analysis

The results were analyzed by Student's t-test using Statistical Package for the Social Sciences (SPSS) version 19. All data were expressed as mean ± SD. p-Value less than 0.05 was considered significant.

Results

Patients with colon cancer were classified into two group according to (age, sex, smoking, treatment with chemotherapy or not, stages of cancer, history of family). Detailed clinic pathological characteristics of the patients are given in (Table 1)

Table 1 The clinical characteristics of patients with colon cancer

Variable	No.	Percentage (%)
Total number of patients	50	100
• Age		
a) (20-40)	16	32
b) (41-70)	34	68
• Gender		
a) Female	21	42
b) Male	29	58
• Family History of cancer		
a) With	32	64
b) Without	18	36
• Stage of tumor diagnosis		
Stage:		
Ⅰ	2	4
Ⅱ	13	26
Ⅲ	31	62
Ⅳ	4	8
• Smoking		
a) Smoker	21	42
b) Non-smoker	29	58
• Disease		
a) Diabetes	25	50
b) Cardiovascular	2	4
c) Asthma	4	8
d) Hypertension	4	8
e) Anemia	50	100
f) Without any disease	25	50
• Obesity		
a) Obese	14	28
b) Non-obese	36	72
• Drug		
a) Chemotherapy treatment	20	40
b) Without treatment	30	60
• Education		
a) Illiterate	9	18
b) Educated	41	82

The result showed decrease of ceruloplasmin activity but no significant variation ($p>0.05$) in sera of colon cancer patients compared with the control group, whereas a highly significant increase ($p< 0.0001$) in concentration of copper and Cu/Zn, and highly significant decrease in concentration of zinc when compared them with the control group (Table 2).

Table 2 Concentrations of parameters under study in patients with colon cancer and control

	Control n=33	Patient n=50	
Parameters	**Mean ± SD**	**Mean ± SD**	**P-value**
Ceruloplasmin (U/L)	126.8 ± 92.6	110.7 ± 86.3	0.4
Zn(µg/dl)	126.69± 70.04	61.48 ± 26.15	0.0001
Cu (µg/dl)	84.3± 44.3	180.98 ± 112.85	0.0001
Cu/Zn	0.75±0.4	3.10±2.7	0.0001

Also the patients were classified according to their cases into four stages, stage 1, 2, 3 and 4. The results revealed no any significant differences ($p>0.05$) between the stages except there was significant differences ($p<0.05$) in the concentration of copper when comparing stage 3 versus stage 4 (Table 3).

Table 3 The level of parameters in all stage groups of patients with colon cancer

	Stage Ⅰ n=2	Stage Ⅱ n=13	Stage Ⅲ n=31	Stage Ⅳ n=4
Parameters	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Cp oxidase (U/L)	46 ± 5.7	138.9 ± 97	97.4 ± 70	155.8 ± 15.6
Zn (µg/dl)	57.5 ± 17.67	54.53 ± 29.54	61.8 ± 25.68	83.25 ± 11.35
Cu (µg/dl)	131 ± 45.25	178.61 ± 113.79	170.1 ± 96.8*	298 ± 201.4
Cu/Zn	2.2±0.09	3.7±3	3.1±2.3	3.5±2.5

*($p<0.05$)

In addition the present study showed highly correlation between Cp and Cu ($r = 0.5$) in patient with colon cancer (Fig 1) whereas there is no any correlation between Cp and Zn ($r = 0.063$) in patients under study (Fig 2).

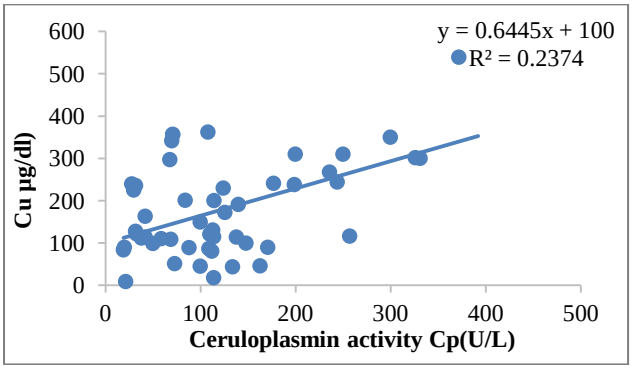

Figure 1 : Correlation between Cp and Cu in patients with colon cancer

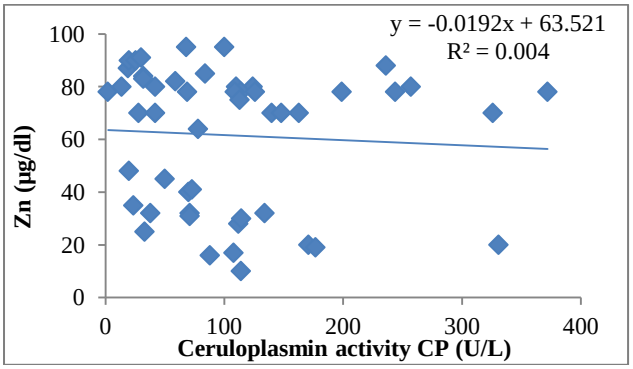

Figure 2: Correlation between Cp and Cu in patients with colon cancer

Discussion

In this study we find decrease in the ceruloplasmin activity of colon cancer patients when compared with healthy control group. This indicated that reactive oxygen species (ROS) can produce oxidative damage to the tissue .The lowered antioxidant capacity or the oxidant-antioxidant imbalance can lead to oxidative damage to cellular macromolecules and leading to cause cancer (Choudhari, *et al.*, 2014). Ceruloplasmin is a blue alpha-2 glycoprotein with a molecular weight of 132,000u, It binds 90-95% of blood plasma copper, has 6-7 copper ions per molecule, and exhibits ferroxidase activity (an iron oxidase), amine oxidase activity, superoxidase activity, as well as it is involved in copper transport and homeostasis (Varela, *et al*., 1997). Ceruloplasmin,were decreased in the head and neck cancer patients suggested that there is an increase in oxidative stress being involved in the pathogenesis of head and neck cancer (Malathi , *et al*., 2011). In other study Ceruloplasmin activity (Cp) showed higher in serum of patients with breast, Lung, gastrointestinal and gynecological cancer compared with the control group (Zowczak, *et al*., 2001). Ceruloplasmin being an effective antioxidant protein, it is one of the acute phase reactants, whose concentration in plasma rises after tissue injury. Most acute phase proteins can be thought of as protecting the organism as a whole, from the possible illeffects caused by the release of free radical oxidation products. This suggests that the organism might respond to ongoing free radical damage by raising the antioxidant efficiency of plasma, by elevated ceruloplasmin levels (Mutalik, 2006; Andrzejewska *et al*., 1992).The increase of ceruloplasmin activity may be associated with enhanced synthesis of ceruloplasmin in the liver as one of the positive acute phase reactant protein (Fleming, *et al.*, 1991) .

In other studies find that Cp protects the erythrocytes of patients with prostate tumors against the oxidative stress promoted by Fe^{2+} and accordingly from the lysis that is induced by metal ions. Furthermore, the results indicate that as antioxidant and ferroxidase Cp is capable to reduce the oxidative stress that develops during the tumor growth (Kotrikadze, *et al.*,2011). (Nayak, *et al.* 2003) found that the determination of specific antioxidants (like ceruloplasmin) and trace elements like Cu may be of value in the early diagnosis of prostate and colon cancer.

In our studies we found that there is a significant increase in the concentration of Cu in patient with colon cancer when compared with control group and this study agreement with other studies. (Adaramoye *et al.* 2010) found that there is an elevated in concentration of Cu in patient with prostate cancer. copper is an essential trace element, playing a critical role in multiple functions in the body ,it has an important role in maintaining good health .When paired with iron, it helps create red blood cells. It is also helps to keep blood vessels, bones, nerves, and the immune system healthy (Rajeswari and Swaminathan, 2014). It is a redox-active transition metal that can participate in single electron reaction and catalyse formation of free radicals. It has an essential element for formation of hemoglobulin, myelin, collagen, and

melanin. It has been suggested to play an important role in several disorders and normal immune function (Wallwork, 1987; Sayır, *et al*., 2011).

Also the present study showed that there is a significant decrease in the concentrations of zinc in patients with colon cancer when compared with the control group and this results agreement with (Dhalla, *et al.*1999). Zinc plays an anti-carcinogenic role by stabilizing the structure of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosome. It is also an essential element to the function of several transcription factors and proteins that recognize certain DNA sequences and regulate gene transcription. Zinc protects cells from free-radical injury therefore the lower level of Zn can also aid in the deterioration of cancer affected on the colon tissue and causing additional damage Al Faris, and Ahmad, 2011; Wu and Sempos, 2004). 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. Zinc 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, 1999).

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