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Research Paper

Antioxidants and Lipids Peroxidation Status 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 caused by many factors such as hereditary, environmental, and dietary factors and insufficiency of physical activity. This study included fifty patients with colon cancer (21 female and 29 male) their ages ranging from 20 to 70 years old. Those patients were enrolled from Al-Hussain Medical City/Kerbala, and 33 healthy subjects who were free from signs and symptoms of cancer their ages were matched with cancer patients as a control group. Reduced glutathione, catalase, malondialdehyde and ceruloplasmin oxidase were measured in sera of those patients and the control group. The results showed a highly significant decrease ($p = 0.0001$) in reduced GSH levels and a highly significant increase ($p = 0.0001$) in the concentration of MDA in sera of colon cancer patients compared with the control group, whereas CAT and Cp oxidase activity did not show a significant difference. The demographic study revealed a significant decrease ($p < 0.001$) in the activity of CAT and a significant increase ($p = 0.05$) in the level of MDA in male patients comparing with the female group. The results of this study indicate that free radicals and antioxidants play an important role in colon cancer.

Keywords: *colon cancer, antioxidants, Glutathione, catalase, ceruloplasmin, lipids peroxidation.*

1. Introduction

Colon cancer is considered one of the leading causes of death in industrialized countries, and it is caused by many factors like hereditary, environmental, and dietary factors and insufficiency of physical activity (Bjorklund, et. al., 2018). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals that are generally known to play a dual role as both deleterious and beneficial molecules. The production and accumulation of ROS/RNS from endogenous or exogenous sources are termed as oxidative stress, and they are present in many types of tumor cells that are linked with exchanged redox regulation of cellular signaling pathways. Oxidative stress causes the imbalance of cellular redox, which has been found to be existent in various cancer cells compared with normal cells (Gupta, et. al., 2014). Glutathione (GSH) has a significant function in the enzymatic system responsible for maintaining intracellular redox balance (Granner, et. al., 2005). Lipid peroxidation by ROS/RNS can cause the damage of cell membranes and this lead to cell death (He, et. al., 2017). Cells can protect themselves against oxidative stress using both enzymes and non-enzymatic antioxidants (Skrzydlewska, et. al., 2005). Lipid peroxidation and antioxidants were studied in a different type of cancers. Subramanyam, et.al (2013) showed that decrease the antioxidant activity and increase the level of lipid peroxidation in a patient with cervical cancer. In vivo study Harris, et. al. (2015) showed the role of glutathione and antioxidants in tumor progression and death of the cancer cells. Others study, Calaf, et.al. (2018) explain how the antioxidants prevent cancer. The aim of the study is the association of colon cancer with dangerous oxidative stress and confirm that gradual imbalance of oxidative/anti-oxidative is followed by the development of colon cancer.

2. Materials and Methods

Patients and control: A case-control study included 50 patients (21 female and 29 male) with colon cancer were enrolled from Al-Hussain Hospital /Kerbala, over a period from October 2013 to March 2014 their ages ranged from 20 to 70 years old and 33 healthy individuals with no history of gastrointestinal illnesses their ages matched with patients group. All patients were subjected to a personal interview using a specially designed questionnaire to assess their health history (age, sex, BMI, family history of cancer, smoking, education).

Specimen collection and preparation: Five milliliters of venous blood were drawn from each patient, allowed to clot at room temperature, and centrifuged at 3000 xg for 15 minutes, and then serum was separated and stored at -70℃ until analysis.

Biochemical assays: The level of reduced glutathione (GSH) was determined as described by Ellman (1959). 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) is a disulfide chromogen and a sulfhydryl group of GSH reduced it to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm on a spectrophotometer and is directly proportional to the GSH concentration. malondialdehyde (MDA) levels were measured spectrophotometrically using the modified procedure of Hassani (2015).Determination of MDA is depending on the reaction of MDA with thiobarbituric acid (TBA) which forms MDA-TBA2, which absorbs at wavelength 532 nm. Catalase activity (CAT) was determined as described by Gòth (1991). The absorbance of hydrogen peroxide was measured at 405 nm by using a spectrophotometer. The enzymatic activity of serum ceruloplasmin (Cp) oxidase was assayed using a o-dianisidine dihydrochloride as substrate as described by Schosinsky et al. (1974).

Statistical analysis:The data of this study were expressed as mean ± SE. Statistical analysis was performed using Student's t-test. P-value ≤ 0.05 was considered significant.

3. Results and Discussion

Patients with colon cancer were classified according to age, sex, education, smoking history, familial history of cancer, stage of their colorectal cancer, and whether they received chemotherapy. Detailed clinical pathological characteristics of patients are given in Table 1.

This study revealed a highly significant decrease ($p < 0.0001$) in reduced GSH levels and a highly significant increase ($p < 0.0001$) in the concentration of MDA in sera of colon cancer patients compared to the control group. CAT and Cp oxidase activity did not show significant differences in sera of patients compared with the control group (Table 2).

Patients were classified into four stages according to the development of the disease. The results demonstrated a highly significant level ($p < 0.001$) of reduced GSH between stage I and stage II of colorectal cancer. There were also significant decreases in the level of GSH in stages II, III, and IV when compared with the control group. There was also a significant decrease ($p < 0.001$) in the activity of CAT compared with control group in stages I and IV. In all stages, MDA levels were significantly increased ($p < 0.001$) compared with the control group, whereas the activity of Cp oxidase was significantly lower ($p < 0.001$) in stage I compared with the control group (Table 3).

Sex differences in redox factor levels were also analyzed. The results revealed a significant decrease ($p = 0.001$) in CAT activity and a significant increase ($p < 0.05$) in the concentration of MDA in male patients compared to females, whereas there were no significant differences in the concentrations of GSH or Cp oxidase (Table 4). Additionally, patients were classified into two groups according to their age, with Group 1 ranging from 20 to 40 years old (32%) and Group 2 ranging from 41 to70 years old (68%). The results showed no significant difference in the level of the parameters under study between the two groups (Table 5).

By using Pearson's correlation coefficient, the results revealed a significant negative correlation ($r = -0.3$, $p = 0.02$) between BMI and the activity of catalase CAT, and a significant positive correlation between BMI and MDA ($r = 0.3$, $p = 0.03$), and between BMI and Cp oxidase ($r = 0.28$, $p = 0.04$) (Table 6).

GSH was found to be lower in patients with colon cancer compared with the healthy subjects. This result is consistent with a previous study (Grubben, et. al., 2006), and another that showed GSH was also lower in patients with liver and breast cancer (Arslan, et. al., 2014). This occurs when free radicals and other oxidative agents in the biological system deplete GSH and outpaced GSH synthesis (Alam, 2014). The elevated levels of GSH cause the rise of antioxidant capacity and the impedance to oxidative stress as observed in many cancer cells (Traverso, et. al., 2013). In this study, the CAT did not significantly vary between the patients and the control group and this result agrees with Woźniak et al. (2012).

In the present study, the levels of MDA were higher in patient groups. This result is matched with the previous study which reported an increase in the level of MDA in patients with colorectal cancer (Skrzydlewska, et. al., 2005). The increasing of oxidative stress is due to lipid peroxidation and cancerous cells which generating free radical to a greater extent (Ramya, et. al., 2011). This study showed a non-significant decrease in Cp oxidase activity between patients and the control group. This indicates that ROS can cause oxidative injury to the tissue. The imbalance of the oxidant-antioxidant can lead to oxidative damage and injury to cellular macromolecules, ultimately resulting in cancer (Choudhari, et. al., 2014).

The increase in oxidative stress, in conjunction with membrane damage due to lipid peroxidation, can lead to compensatory increases in the production of antioxidant enzymes that scavenge oxygen radicals. Antioxidant enzymes are intracellular, whereas non-enzymatic antioxidants are present extracellularly and intracellularly, and they are existent at the site of generation and at the site of action of ROS in a sufficient amount. Their action as chemical scavengers, they prevent cellular damage which induced by free radicals (Manoharan, et. al., 2005).

The GSH concentration was significantly lower ($p < 0.05$) in all stages of colon cancer in comparison with the control group. Other study showed that the levels of GSH were reduced in the advanced stages of oral cancer when compared with the initial stages (Azouzi, et. al., 2017). The usages of GSH or excessive ROS in circulation generated by the tumor are the most probable explanations for the depletion of GSH in the plasma of cancer patients.

In the present study, the level of CAT was variable in all stages of colon cancer, whereas Negahdar et al. (2005) found that CAT levels were decreased in all stages of breast cancer with no effect between stages suggesting high free radical production. CAT activity was significantly lower in oral cancer patients compared with healthy subjects, and gradually its activity reduced from stage II to stage IV in oral cancer patients (Azouzi, et. al., 2017). CAT activity of patients with advanced cancers of various tissues appeared to be lower than that of patients with early malignant diseases (Gönenç, et. al., 2005).

The increased concentration of MDA in all stages of colon cancer compared with the control group is similar to the study of Gönença et al. (2005) which shows that ROS are increased in patients with breast cancer (Kosova, et. al., 2014). One study showed the elevated level of MDA and decrease non-enzymatic and enzymatic antioxidants in oral cancer patients comparing with healthy subjects. The MDA levels gradually increased, whereas antioxidants gradually decreased from stage II to stage IV of oral cancer patients (Azouzi, et. al., 2017). High levels of lipid peroxidation products have been detected in patients with early and advanced cancers across different cancers compared to healthy as a control (Kosova, et. al., 2014), however, Gopčević et al. (2013) found that lipid peroxidation, as assessed by MDA levels, was not significantly different between stages of colorectal carcinoma.

The activity of Cp oxidase in the present study was increased in patients with stage II and with late-stage colon cancer patients compared with the control group. This result in agreement with the results of Upadhya et al. (2002) in stages of cervical carcinoma, whereas Arumanayagam et al. (1993) found that Cp oxidase increased only in advanced stages of cervical carcinoma.

In this study, the percentage of males with colon cancer was higher than for females. This variability may be at least in part due to hormonal differences. In addition, weight, blood pressure, menopause and the intake of some drugs can modify the antioxidant enzyme activities (Saraymen, et. al., 2003). The non-significant difference in the parameters under study are in agreement with Özbay and Dülger (2002), They showed that age has no effect on serum MDA and GSH levels in females, whereas, in males, there was an increase in serum GSH level but not in MDA level at age group (25-45) years compared to age group (46-70) years.

Obesity is considered one of the risk factor for many diseases. In chronic obesity, sources of the antioxidant enzymes become minimize, resulting in a reduced ability to manage oxidative stress. Obesity is one of the causes of the low activity of the cytoprotective enzymes in human, which may lead to atherosclerosis, cancer and other diseases (Gallagher and LeRoith, 2015).

The negative correlation between CAT activity and BMI was similar to the study of Rupérez et al. (2013), who found decreased CAT was significantly correlated with obesity and insulin resistance biomarkers in childhood obesity.

This study found the activity of Cp oxidase was correlated with BMI in patients with colon cancer, whereas another study by Safavi et al. (2012) found that Cp oxidase is not associated with BMI in obese patients. Also, Arner et al. (2014) have shown that Cp oxidase is overexpressed in the adipose tissue of obese cancer patients, suggesting that its expression and secretion are increased

in obesity and it is a major contributor to the circulating Cp oxidase level (Arner, et. al., 2014).

Table 1: The characteristic features of colon cancer patients.

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

Table 2: Parameters under study in colon cancer patients vs. control

	Control n = 33	Patient n = 50	
Parameters	Mean ± SD	Mean ± SD	P-value
GSH (µmole/L)	231.6 ± 192.3	76.6 ± 11.5	0.0001
CAT (KU/L)	108.8 ± 73.56	107.3 ± 91.4	0.9
MDA(µmole/L)	7.6 ± 7	16 ± 10.8	0.0001
Cp oxidase (U/L)	126.8 ± 92.6	110.7 ± 86.3	0.4

Table 3: Parameter levels for each stage of colon cancer and control

	Stage Ⅰ n = 2	**Stage Ⅱ n = 13**	**Stage Ⅲ n = 31**	**Stage Ⅳ n = 4**	**Control n = 33**
Parametes	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
GSH (µmole/L)	128 ± 13.6*	36.5±34.6**	92.8 ± 14**	55 ± 5 **	231.6±192.3
CAT (KU/L)	63.35 ± 9**	104.76 ± 11	118.3 ± 86	52.4±8**	108.8±73.56
MDA (µmole/L)	16 ±12**	16 ± 13**	16 ± 9**	17 ±14**	7.6 ± 7.5
Cp oxidase (U/L)	46 ± 5.7**	138.9 ± 97	97.4 ± 70	155.8±15.6	126.8 ± 92.6

*****($p < 0.001$) stage I vs. stage II
******($p < 0.001$) stage II I vs . IV

Table 4: Parameter levels in sera of male and female patients with colon cancer

Parameters	Male Mean ± SE n = 29	Female Mean ± SE n = 21	p-Value
GSH (µmole/L)	86 ± 6	63.6 ± 4	NS
CAT (KU/L)	79.3 ± 5	146 ± 10	0.001
MDA (µmole/L)	18.6 ± 1	12.4 ± 1	0.04
Cp oxidase (U/L)	121.3 ± 11	96.2 ± 7	NS

Table 5: Parameter levels in sera of patients with colon cancer separated by age

	Age (20-40) n = 16	Age (41-70) n = 34	
Parameters	Mean ± SE	Mean ± SE	p-Value
GSH (µmole/L)	63.85 ± 5	82.6 ± 5.5	NS
CAT (KU/L)	94.6 ± 8	113.3 ± 10	NS
MDA (µmole/L)	17 ± 1.2	15.4 ± 1.1	NS
Cp oxidase (U/L)	89 ± 3	121 ± 11	NS

Table 6: Correlation between BMI (kg/m^2) and parameters under study in patients with colon cancer

Parameters	r	p-Value
GSH (µmole/L)	0.116	NS
CAT (KU/L)	-0.3	0.02
MDA (µmole/L)	0.3	0.04
Cp oxidase (U/L)	0.28	0.04

4. Conclusions

Colon cancer is correlated with serious oxidative stress. Lipid peroxidation and antioxidant status are associated with the progression of colon cancer.

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