Levels of Interleukin-6 and Certain Antioxidants in Patients with Colorectal Cancer

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ABSTRACT:

BACKGROUND:

Colorectal cancer (CRC) is one of the most aggressive common tumors in humans. It is known that development and progression of tumors is associated with deviations in the human immune system**.**Interleukin-6 (IL-6) is a pro-inflammatory and anti-inflammatory cytokine that it is secreted by T cells and macrophages to stimulate immune response, during infection and after trauma**.** Antioxidants catalase (CAT), Glutathion (GSH), Ceruloplasmin (CP) and Malondialdehyde (MDA) are substances that when present at low concentrations compared to those of an oxidizable substrate significantly delays or inhibits oxidation of that substrate**.**

OBJECTIVE:

The main objective of this study is to determine the frequency of IL-6, CAT, GSH, CP and MDA in patients with CRC.

PATIENTS & METHODS:

This study included 40 healthy control donors (20 male and 20 female) and 60 patients with CRC (20 smokers male, 20 non smokers male and 20 non smokers female). They were obtained from the people attending the out Patient Clinic/ Baghdad Teaching Hospital in Medical City. Ages ranges were 40-60 years and matched by age and sex to healthy control donors with mean age 53.24 ± 2.32 years

RESULTS:

The mean values of IL-6 and CP in sera of patients (smoker and nonsmoker groups) with CRC were significantly increased as compared to healthy control donors ($P<0.01$) and the mean values of CAT, GSH and MDA in sera of patients (smoker and nonsmoker groups) with CRC were significantly decreased as compared to healthy control donors ($P<0.01$) and results analysis shows that the IL-6, CAT, GSH, CP and MDA of female with CRC are less than of a male.

CONCLUSION:

It is clear from this study that there is a relationship between the levels of IL-6, GSH, CAT, CP, and MDA concentrations and CRC patients and may be a good indicator to evaluate this disease.

KEY WORD: interleukin-6, catalase, glutathione, ceruloplasmin, malondialdehyde and colorectal cancer

INTRODUCTION:

Colorectal carcinoma is one of the most aggressive common tumors in humans. It is known that development and progression of tumors is associated with deviations in the human immune system. [(1)]. Colorectal cancers ranks third in frequency in men and second in women. Various factors have been shown to be responsible for the accumulation of mutations in CRC including inheritance and environmental factors [(2)]. IL-6 contributes to a multitude of physiological and pathophysiological processes [(3)]. Different type of cells is produce IL-6, *in vivo* the main sources are stimulated fibroblasts, monocytes, and endothelial cells. Macrophages, T-cells and B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells, and keratinocytes also produce IL-6 after stimulation. Many research suggest that IL-6 produced by the tumor cells acts as a growth factor [(4)] and acts as both a pro-inflammatory and anti-inflammatory cytokine that it is secreted by T cells and macrophages to stimulate immune response, during infection and after trauma, especially burns or other tissue damage leading to inflammation, in terms of host response to a foreign pathogen during infection. IL-6 is also a "myokine," a cytokine produced from muscle, and is elevated in response to muscle contraction [(5)]and significantly elevated with exercise, and

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precedes the appearance of other cytokines in the circulation [6]. An antioxidant is a substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or inhibits oxidation of that substrate [7]. Catalase (Cat, Hydrogen peroxide oxidoreductase) is a metalloprotein present in all oxygen metabolizing cells. Catalase activity in bloods responds to the variation in sex, but it is not affected by age [8]. Glutathione is the important of nonprotein cellular thiol compound, exists in most body organism like: liver, kidney erythrocyte, brain [9]. Reduction of GSSG to two moles of GHS is the function of glutathione reductase, an enzyme that requires coupled oxidation of NADPH[10]. Glutathione peroxidase exerts a protective function for hemoglobin and other SH – proteins and the detoxification of xenobiotics, carcinogens, free radicals, and peroxides; regulation of immune function; and maintenance of protein structure, function and turnover, as it acts as reductant in oxidation – reduction processes and also serves as a reservoir of cycteine and believed that glutathione is important to determine the resistance to cancer chemotherapy and therefore measurement of glutathione (GSH) in tumor tissue is clinical relevance[11]. Human ceruloplasmin is a metalloprotein contain (90 to 95%) of plasma copper. Different physiological roles played by ceruloplasmin including, scavenging of superoxide anion radical's ($O^{\bullet}_2$), through this activity ceruloplasmin acts as a major extracellular scavenger that performs the same role-played by the SOD, and plays another role through preventing the formation of free radical intermediates or peroxide that might be generated by nonenzymatic (non – CP) oxidation of Fe (II) complexes [12]. Cells possess the capacity to generate huge amounts of ROS and ROS induce lipid peroxidation, a chain process which affects unsaturated fatty acids mainly localized in cell membranes, in which end product as malondialdehide (MDA) is generated. MDA, which is itself responsible for some of the damaging effects of free radicals on DNA and cell membranes. Lipid peroxidation products diffuse from the site of inflammation and can be measured in the blood [13].

MATERIALS AND METHODS:

This study included 60 patients with CRC (20 smoker male, 20 nonsmoker male and 20 nonsmoker female) with histologically proven colorectal carcinoma, they were obtained from the people attending the out Patient Clinic/ Baghdad Teaching Hospital in Medical City, then matched by age and sex to 40 healthy control donors (20 male and 20 female). The laboratory and histologically tests were done in Teaching Laboratories of the Medical City and the Department of Biochemistry / College of Medicine University of Baghdad. All subjects were evaluated by BMI (weight in kilograms divided by the square of height in meters). Subjects were excluded if they showed medication or had any evidence of metabolic disease other than obesity. Blood samples were taken from donor individuals in all groups for estimating IL-6, CAT, GSH, CP and MDA concentrations. The IL-6 was determine by the IL-6 Kit that it is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) from HUMAN-Germany [14]. The method of Aebi was used to determine the erythrocyte catalase activity. In the ultraviolet range H_2O_2 shows a continual increase in the absorption with a decrease in wavelength. GSH was estimated by the method of Beutler et al using dithiobisnitrobenzoic acid (DTNB). The oxidase activity of ceruloplasmin was measured according to the modified method using P-phenylene diamine – 2HCl (PPO-2HCl) as a substrate. Malondialdehyde was determined by measuring thiobar-bituric reactive species using the method of Ruiz-Larrea et al. [15].

RESULTS:

The results presented in table 1 revealed that there is no significant difference in age and BMI between health control donors and CRC patients.

Parameters	Healthy control donors (n=30) Mean ±SD	CRC patients (n=60) Mean ±SD	*P-value*	Significant
Age	53.24 ± 2.32	50.15±3.10	0.39	NS
BMI (kg/m^2)	25.94 ± 0.34	26.13 ± 0.29	0.21	NS

The characteristics of 60 CRC patients and 40healthy control donors are shown in table 1. There was a significant difference in mean values of IL-6, CAT, GSH, CP and MDA levels in the patients with CRC ($P < 0.01$) as compared to the healthy control donors as shown in table 2.

Table 2: Levels of IL-6, CAT, GSH, CP and MDA patient with CRC and healthy control donors.

Groups	No.	IL-6 (pg/ml) Mean ± SD	CAT (U/mg/Hb) Mean ± SD	GSH (µmol/L) Mean ± SD	CP (IU) Mean ± SD	MDA (µmol/L) Mean ± SD
Male:	60					
Control	20	4.14±1.98	158.695±14.564	0.789 ± 0.075	44.148 ± 1.653	9.838±0.981
Patient nonsmokers	20	9.25± 4.93	91.154 ± 17.832	0.161 ± 0.062	56.617 ± 3.042	7.110 ± 0.951
Patient smokers	20	10.012±3.89	81.352 ± 15.236	0.128 ± 0.041	58.588 ± 3.658	6.73 ± 0.71
Female:	40					
Health control donors	20	4.024±1.56	152.542±13.987	0.809± 0.021	42.152 ± 1.632	9.309±0.875
Patient	20	9.832±3.56	92.254± 11.847	0.102 ± 0.078	53.147 ± 3.982	7.084 ± 0.828

DISCUSSION:

Results presented in table 2 revealed that the mean values of IL-6 and CP in sera of patients (smoker and nonsmoker groups) with CRC were significantly increased as compared to healthy control donors (P<0.01) and the mean values of CAT, GSH and MDA in sera of patients (smoker and nonsmoker groups) with CRC were significantly decreased as compared to healthy control donors (P<0.01) and results analysis shows that the IL-6, CAT, GSH, CP and MDA of female with CRC are less than of a male, that is IL-6 a cytokine plays significant role in the growth and differentiation of cells. Several studies have demonstrated that the role of IL-6 in tumor cell growth may depend on the tumor cell type [16]. Human tumor cell lines, including esophageal squamous cell carcinoma, melanoma, lung carcinoma, and also colorectal cancer, have been reported to reduce IL-6. It has been demonstrated that IL-6 acts as a colorectal growth factor and as an autocrine growth factor for colorectal cancer cells [17]. In addition IL-6 induced lymphoblastic tumor igenicity is due possibly to the inhibitory effect on tumor immunity of very high concentrations of this cytokine and that natural killer cell dysfunction induced by IL-6 production from tumor cells is a mechanism of tumor escape from immune surveillance [18]. In the present study, the serum IL-6 level In were significantly higher in patients with colorectal cancer than in healthy control donors, supporting the previous report that patients with metastatic colorectal cancer had significantly elevated serum IL-6 levels compared with healthy control donors. These findings support the hypothesis that the serum IL-6 level reflects the content of IL-6 in the tumor component, that the elevation in the serum level may reflect disease progress [19]. Results of CAT are in agreement with study of Dhalla and study of Dusinovic, the lower of catalase activity in erythrocyte of patients with CRC may be attributed to the formation of free radicals and increase of super oxidizing, the decrease of catalase activity as a result of the tissue damage because of the inhibiting protection function of (Cat, GSH, SOD) and the loss of enzymes from cells [20]. Glutathione plays a crucial role in the detoxification of peroxides, hydroperoxides and other free radicals. A decreased GSH level demonstrates the activation of adaptive mechanisms counteracting an oxidative stress because GST uses GSH as a substrate to detoxify substances, thus inducing oxidative stress [21]. It is generally known that colorectal cancer is associated with oxidative stress and an imbalance in the oxidative/ antioxidative state, which result in disease progression [22]. The GSH level and GST-dependent enzymes have been investigated in blood erythrocytes, tumor tissue, blood serum and plasma [23]. The obtained results in our study agree with a study of Edita Baltruskeviciene1 [24],that the mean ± SD of erythrocyte GSH was a statistically significant decrease in patients with CRC as compared to healthy control donors, the decrease in the GSH levels may be due to the increased turnover of GSH for preventing oxidative damage in these patients. Similar reports of lowered GSH levels in cancers have been reported earlierby Ahmed *et al*. [25] in patients with cervical cancer. Reduced glutathione levels in cancer were also reported by Bhuvarahamurthy *et al*. and Faber *et al*. An increase in tumour tissue glutathione levels in oral squamous cell carcinoma has been reported by Subapriya *et al*. [26]. These results are in agreement with Cunnigham et. al. that several mechanisms determining CP role as antioxidant

have been suggested, the first mechanism is that CP prevents decompartmentised iron from acting as free radical catalyst since its rapidly catalyzes the oxidation of Fe (II) (which is present in intracellular) ferritin into Fe (III) prior to its incorporation into apotransferrin.

In general, the major antioxidant action of CP is to bind copper ions in a form that will not stimulate free radicals. At last, ceruloplasmin is considered now as one of the positive acute phase reactant whose concentration increases upon different diseases (Burtis and Ashwood) [27].In our study lipid peroxidation product i.e, malondialdehyde (MDA) has been decreased in the erythrocytes of patients with colorectal cancer. Studies have reported similar findings in patients with breast cancer [28]. They have also observed a decrease in plasma MDA with tumor size and progression. A similar decrease in tissue lipid peroxide was observed in oral squamous cell carcinoma [26]. Saroja*et al*. have also reported diminished lipid peroxidation in oral tumor tissue and a decrease in phospholipid content and an increase in cholesterol: phospholipid ratio. Skrzydlewska et al have reported increased activity of superoxide dismutase, glutathione peroxidase and glutathione reductase (enzymatic oxidant defence system) and a decreasein GSH content (non-enzymatic antioxidant parameter) in cancer tissue suggesting an increased defense against oxidant damage in cancer [29].

CONCLUSION:

It is clear from this study that there is a relationship between the levels of IL-6, GSH, CAT, CP, and MDA concentrations and CRC patients and may be a good indicator to evaluate this disease.

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