Evaluation of CEA,CA19-9, and CA242 Tumor Markers in Patients with Colorectal Cancer by ELISA Technique

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Abstract

Colorectal cancer (CRC) is one of the common malignant tumors of gastrointestinal tract (GIT). A total of 40 patients with CRC were studied preoperatively and postoperatively while only 20 healthy persons were studied. Their ages ranged between 18-78 years.This study was carried out to investigate the possible association of some risk factors with CRC such as (age, gender, smoking, inflammatory bowel disease, and ulcerative colitis).

With application of ELISA technique, Serum levels of CEA, CA 19-9, and CA 242 was measured preoperatively and postoperatively. The results showed an increase in preoperative serum level of CEA in CRC patients when compared to both postoperative and control groups, There was a significant increase in the serum level of CEA (10.73 ± 4.01) comparison with the two other groups (postoperative 3.95 ± 1.55) and control (1.91 ± 0.27) at ($P<0.05$), but no significant differences between the two groups (postoperative and healthy) which have mean (3.95 ± 1.55 and 1.91 ± 0.27) respectively. Also showed a significant increase in serum level of CA19-9 in patients preoperative (23.28 ± 1.86), who were differed significantly from the mean of serum in patients postoperative (18.63 ± 1.40) and control group (19.11 ± 1.46) at ($P<0.05$), When compared the serum level of CA242 revealed a significant increase in serum level of patients preoperative (19.58 ± 3.46) as compared to postoperative(8.75 ± 1.77) and control group(9.85 ± 2.22) at ($P<0.05$).

Key words: *Colorectal cancer, Biomarkers, Metastasis, CEA, CA19-9, CA242.*

Introduction

Cancer is a disease that occurs when control is lost on cell division and growth as well as on the metastasis of abnormal cells. Reasons for cancer are both intrinsic, i.e. infections, smoking, radiant sources, and extrinsic, i.e. genetic and metabolic mutations, along with abnormal immune responses and hormone levels [(1).]

Cancers start in the cells that line the inside of colon (the longest fragment of the large intestine) and rectum (occupies the last several inches prior to the anus) [(2).] Stool blood, altered movement of bowel, continuous tiredness, nausea, vomit, malaise, anorexia, abdominal distension and losing of weight are among the main symptoms of CRC [(3).] Ageing and disturbed life style were reported to be the major reasons for developing CRC, with genetic abnormalities affecting only minor cases [(4).] Dietary influences which contribute to higher are red meat which is over processed, alcohol consumption, IBD (inflammatory bowel disease) [(5).] Tumor develops under strong influences from genetic and epigenetic disorders, while the survival can be best prognosis based on immunological microenvironment [(6).]

Materials and Method

Blood samples (5 ml) were collected from patients with colorectal cancer (preoperative- postoperative) healthy persons (control) by using plastic syringes. Serum samples were prepared by centrifuging the blood (5000 rpm for 15 min).

Tissue Biopsies: Biopsies were collected from tissues of CRC patients, followed by fixation in buffered formalin (10 %), embedding in paraffin wax, and finally staining by using hematoxylin-eosin. Tissue samples were collected from histopathological laboratories of Gastroenterology and Liver Diseases Teaching Hospital, Baghdad.

Immunological Assessments:Enzyme Immunoassay for Determination of Serum Tumor Markers by Using ELISA Kit: Quantitative determination of serum levels of three tumor markers (CEA, CA19-9 and CA 242) in both patient and control groups was performed by means of ELISA (Enzyme Linked Immunosorbent Assay) .

Results

The study included 40 patients with colorectal cancer, 23 male (57.50%) and 17 female (42.50%), with an age range of 18-78 years .The selected patients were classified into two groups: the first group represented serum of preoperative patients and the second group was of serum of postoperative patients. These two groups were compared to the third healthy group which was used as control. The control subjects included 20 individuals, 11 males and 9 females with ages ranged between 24 and 58 years.

Distribution of patients with colorectal cancer according to different risk factors:

The age of the 40 adult CRC patients included in this study ranged between 18 and 78 years, the maximum numbers of colorectal cancer patients was found within age group more than 60 years (20: 50%), then 12 patients in the age group of 40-60 years (30%), and the minimum number was 8 patients in the age group of less than 40 years (20%). Results in Table (1) demonstrate that the total number of patients was 40, of which 23 were male (57.50%) and 17 were female patients (42.50%). This implies that colorectal cancer is more common in men than in women, with significant differences between the two genders at $P<0.05$, also the proportion of smoking included 21 smoker patients (52.50%) and 19 non- smoker patients (47.50%), with statistically non-significant difference.

Carcinoma groups grading of the present study revealed that well- differentiated adenocarcinoma (G1) was seen in 4 cases (10.00%) of the CRC group, while 30 cases (75.00%) of patients had moderate differentiated adenocarcinoma (G2), and 6 cases (15.00%) had poorly differentiated adenocarcinoma (G3).There were a significant differences at ($P<0.05$) among carcinoma groups according to their grading (Table 1).

In the current study, most of the patients had carcinoma as the type of colorectal cancer. Concerning the staging of the tumor, 6 patients (15.00%) had tumor that invaded the submucosa (T1), 12 (30.00%) had tumor that invaded the muscularis (T2), and 22 (55.00%) had tumor that invaded through the mascularis into the pericolorectal tissues (T3).

The results of our study showed that, among the 40 patients with CRC, 4 (10.00%) had genetic disease, 9 (23.00%) had polyp, 11 (27.00%) had ulcerative colitis, and 16 (40.00%) had diabetes mellitus type II. There were a significant differences at ($P<0.05$) among carcinoma groups according to the risk factors. Most of tumors are localized in different sites of colon. In this study, about 2 patients (5.00%) had tumor in the transverse colon, 3 (7.5%) in the right colon, 8 (20.00%) in the sigmoid colon, 12 (30.00%) in the rectum, and 15 (37.5%) in the rectosigmoid.

Serum Levels of CEA, CA19-9, and CA242 Tumor markers in patients with CRC According to their characteristics and risk factors:

Results in Table (32) exhibited a significant increase of serum levels CEA in patients with age range of 40-60 years as compared with those with age less than 40 years old. However, there were no significant differences between the serum levels of CEA in patients with age more than 60 years old and the other two groups at $P<0.05$.

Comparing serum levels of CA19-9 showed no significant effects of age in patients with age less than 40 years old, 40-60, and more than 60 years old.

Significant increases were recorded in serum levels of CA242 in patients with age range of 40-60 years as compared with serum levels in patients with age less than 40 years old and more than 60 years old at $P<0.05$. Also, the patients with age more than 60 had a significant increase in serum levels of CA242 in comparison with patients with age less than 40 years old at ($P<0.05$).

The results in table (2) show a significant increase in the serum level of CEA in females in comparison with that in males at $P<0.05$. Nevertheless, no significant differences were recorded in the serum levels of CA19-9 and CA242 between males and females. Also the results demonstrate a significant increase in serum level of CEA in smoker patients as compared with non- smoker patients at $P<0.05$. The statistical analysis showed non-significant difference in the serum levels of CA19-9 (and CA242 between smoker and non-smoker patients.

The results showed no significant difference in sera mean levels of CEA, CA19-9, and CA242 in patients with colorectal cancer in the T1, T2 and T3. while the relationship between serum levels of CEA and tumor grades of patients with colorectal cancer showed a significantly increased mean level of CEA in patients with G2 tumor as compared with G1 and G3 at P<0.05. There was no significant difference in mean levels of CEA between G1 and G3. The statistical analysis also showed non- significant difference in serum means levels of CA19-9 and CA242 in patients with G1, G2, and G3 (Table 2).

Table 1. Distribution of sample study of patients according to difference risk factors:

Factors		**Percentage (%)**	**P-value**
Age group (year)	Less than 40	8(20%)	0.0001 *
	40-60	12(30%)	
	More than 60	20(50%)	
Gender	Male	23 (57.50%)	0.0372 *
	Female	17 (42.50%)	
Smoking	Smoker	21(52.50%)	0.094 NS
	Non-Smoker	19 (47.50%)	
Grade of Tumor	Grade I	4 (10.00%)	0.0001 *
	Grade II	30 (75.00%)	
	Grade III	6 (15.00%)	
Stage of Tumor	Stage I	6(15.00%)	0.0001 *
	Stage II	12(30.00%)	
	Stage III	22(55.00%)	
Other diseases	Genetic disease	4 (10.00%)	0.0001 *
	Polyp	9 (23.00%)	
	Ulcerative colitis	11 (27.00%)	
	Diabetes mellitus type II	16(40.00%)	
Site of Tumor	Transverse colon	2 (5.00%)	0.0001 *
	Right colon	3(7.5%)	
	Sigmoid colon	8 (20.00%)	
	Rectum	12 (30.00%)	
	Rectosigmoid	15 (37.5%)	
* (P<0.05), NS: Non-Significant.			

Table 2: Serum Levels of CEA, CA19-9, and CA242 Tumor markers in patients with CRC According to their characteristics and risk factors:

Studied groups	Mean ± SE		
	CEA in serum(ng/ml)	CA 19-9 in serum(U/ml)	CA 242 in serum(U/ml)
Less than 40	2.88 ± 0.14 b	17.52 ± 2.04 a	4.16 ± 1.35 c
40-60	10.48 ± 5.67 a	25.09 ± 2.69 a	20.63 ± 4.64 a
More than 60	6.74 ± 2.43 ab	19.91 ± 1.37a	12.85 ± 2.34 b
LSD value	5.483 *	9.947 NS	6.808 *
P-value	0.0330	0.171	0.0457
Male	2.62 ± 0.34 b	21.68 ± 1.46 a	15.07 ± 2.47 a
Female	13.72 ± 4.92 a	19.98 ± 1.98 a	12.95 ± 3.43 a
LSD value	8.341 *	4.849 NS	8.194 NS
P-value	0.0098	0.488	0.608
Smoker	11.36 ± 4.04 a	20.94 ± 1.87 a	15.14 ± 3.04 a
Non-smoker	2.89 ± 0.43 b	20.97 ± 1.42 a	13.09 ± 2.66 a
LSD value	1.993 *	4.801 NS	8.112 NS
P-value	0.044	0.986	0.615
T1	2.56 ± 0.57 a	21.93 ± 2.72 a	16.70 ± 5.63 a
T2	10.92 ± 5.72 a	22.57 ± 2.74 a	17.67 ± 4.58 a
T3	6.69 ± 2.42 a	19.81 ± 1.38 a	11.57 ± 2.25 a
LSD value	8.993 NS	6.729 NS	11.370 NS
P-value	0.319	0.610	0.478
G1	3.53 ± 1.09 b	23.22 ± 2.84 a	17.20 ± 4.45 a
G2	9.50 ± 3.17 a	20.17 ± 1.49 a	12.46 ± 2.48 a
G3	3.03 ± 0.27 b	21.84 ± 2.69 a	18.30 ± 5.66 a
LSD value	5.993 *	7.288 NS	12.314 NS
P-value	0.0420	0.566	0.368

*(P<0.05), NS: Non-Significant
Means with different letters in same column differed significantly

Discussion

The development of colorectal cancer is caused by a combination of genetic and environmental factors. Epidemiology studies have revealed a number of risk factors for colorectal cancer including age, gender, smoking, family history of colon cancer or inflammatory bowel disease. Various individual characteristics or behaviors are included among the risk factors since they enhance the possibility to develop CRC. Age is one of the major risk factors for CRC. The risk of CRC increases with age, Several hypotheses have been suggested to find explanations for such a correlation between susceptibility to cancers and the progression in age, ageing of individuals is associated with increased and accumulated exposure to carcinogenic materials from the environment, In addition, the cellular mutation repair ability can be declined with increasing age [7].

Our study demonstrated that CRC is more common in men than in women. The incidence and mortality rates are higher by about 30 and 40%, respectively, in men as compared to women, while full explanation for this gender differences is not yet available, it is believed to be due to several factors such as higher exposure to carcinogens , differences in sex hormones, in addition to the interactions among the different factors [8].

Also the proportion of patients who smoked was higher than non-smokers; many studies have reported a higher risk of CRC among cigarette smokers, especially among those with a long history of smoking (9). Smoke from tobacco was reported to be one of the main carcinogen sources, such as heterocyclic amines, polycyclic hydrocarbons and nitrosamines. However, the evidence about the roles of these materials in development of colon cancer is insufficient (10).

Patients with adult onset type 2 diabetes show greater risk of CRC [11]. Despite that the two diseases have several risk factors in common, such as obesity and a sedentary lifestyle, the correlation between them remains even after accounting for physical activity, body mass index, and waist circumference [12]. Patients with chronic inflammatory bowel disease (IBD), who commonly suffer from inflammation in colon for extended periods, were reported to show a two-fold higher risk of CRC development [13]. The extent, severity and duration of ulcerative colitis (UC), the most frequently reported form of IBD, were shown to associate with an elevated risk to develop cancer , UC is a chronic IBD with an unknown reason, mainly influencing the large intestine mucosa [14]. Family risks of CRC are of a greater risk of developing the disease, with that history is mostly driven by genetic mutations and environmental factors [15]. Genetics contributes to CRC risk by both gene-regulated pathways directly involved in disease development and inherited mutations accounting for about 10% of cases [16]. The polyp is the classical model of colorectal cancer pathogenesis and it describes the phases of transition from benign tumors into colorectal cancer over many years, primary factors of polyp to CRC sequence are gene mutations, epigenetic alterations and local inflammatory changes [17].

Tumor markers:

Biochemical analyses are helpful to diagnose and to deal with cancer cases.,Various tumor markers have been studied with respect to gastrointestinal cancer, including (**CEA**, **CA 19-9**, and **CA 242**).

CEA (carcinoembryonic antigen):Is a cell surface glycoprotein that is produced in the GI tract and pancreas in the prenatal period and released to the blood, CEA belongs to a group of molecules known as carcinoembryonic protein [18]. Clinical studies indicate that the cancer antigen (CEA) is associated with the development of metastases in colorectal cancer, although the biological function of CEA is not fully understood [19]. Higher CEA concentrations can be also detected in a number of benign and non-neoplastic cases such as inflammations of the lung, liver, and colon.

Conclusions

1. In our study, we found that elderly age, male gender, smoking, and other infection, appeared to be the most possible association factors for colorectal cancer.

2. The appearance of tumor marker and their concentration are related to the genesis and growth of malignant tumors in patients, and it should correlate with tumor stages and grades.

3. There was a significant elevation in mean levels of CEA, CA19-9, and CA242 serum of CRC patients, when compared with healthy subjects.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they

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