Comparative study of CA19-9 levels as tumor marker in sera and tissues of patients with stomach, colon and rectum cancers

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Summary:

Background: CA19-9 is among a group of mucin glycoprotein sialosyl lewis antigen (SLA) having come to be recognized as a circulating cancer associated antigen for gastrointestinal cancer. Basic research into cancer causation will be a powerful determinant of intervention in the transformation process reinforcing the need for developing effective molecular tumor biomarkers for early detection of malignant evolution in tissues of organs i.e. stomach, colon and rectum. In this study, we make a comparsion between the levels of CA19-9 in sera and tissues of patients with these diseases to see if their levels are proportional and if it is a tumor marker that affect with malignant cells in tissues of these organs.

Patients and methods: Carbohydrate antigen 19-9 (CA19-9) levels were measured in sera and tissues' of 8 patients with stomach cancer (G_1), 8 patients with colon cancer(G_2), 8 patients with rectum cancer (G_3) and 30 healthy subjects (G_4), by the enzyme-linked immunosorbent assay (ELISA) technique.

Results: The results of CA19-9 levels in sera were (42.625±33.088; 47.013±0.318 and 10.938±0.979)U/ml for G_1, G_2 and G_3 respectively compared with serum CA19-9 level of G_4, which was 7.74±4.92 U/ml. The results were found to be significantly higher than control group ($p < 0.005$).The results of CA19-9 levels in tissues' homogenate were (1170.25±8.45; 535.75±263.46 and 483.75±16.37)U/ml for G1, G2 and G3 respectively. The results revealed significantly higher levels of CA19-9 in tissue's homogenate of each patients group compared with the serum levels of the same patients ($p < 0.005$).

Conclusions: Normal level of CA19-9 were found in sera of rectum cancer patients compare to the cutoff value 37U/ml in literatures 1,2, but it is significantly higher than our control group. **,** Significant high levels of CA19-9 were found in sera of colon cancer patients. , Sera of stomach cancer patients showed elevation in CA19-9 level in 50% of cases. **,** Significant high levels of CA19-9 were found in tissue's homogenate of rectum, colon and stomach cancer patients compared to its levels there serum.

Keywords: CA 19-9, tumor marker, stomach cancer, colon cancer, rectum cancer.

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Introduction:

Cancer

Carcinogenesis is a multistage process in which normal cells are initiated and then promoted into cancer by creating a clonogenic population of transformed neoplastic cells. In current molecular oncologic thinking, intrinsic cellular oncogenes, growth suppressor genes and the dysregulation of steps that control cell cycle, cell proliferation, and differentiation through gene expression play interactive roles that transform normal cells into a premalignant state and, ultimately, into frank malignancy. 3, 4 . In Iraq, 13985 different cases of cancer estimated during 2002, gastric carcinoma incidence rates was 442 case, 266 of cases are men and 176 are women. The percentage pf gastric carcinoma was 3.16% of all kinds of cancers. 5 Colorectal cancer projected to afflict 559 Iraqi person in 2002 (colon-407-(226men, 181women), rectum-152-(81men, 71women)). The percentage of colon cancer cases was 2.91% and of the rectum cancer cases were 1.09% of total malignant cases. 5

Tumor marker

With new molecular biology technologies and tools, the concept of early detection of cancer is undergoing dramatic transformation. Early clinical detection of asymptomatic cancer, stage T1, NO or stage I, without evidence of nodal or hematogenous spread, is increasingly common. 6 Tumor markers can indicate the presence of small foci active tumor that cannot be detected by currently available imaging techniques. Also, the short half-life of the tumor markers facilitates their use in assessing changes in tumor burden during therapy. Furthermore, the assays are safe, reliable and relatively cost efficient. 7 Serum carbohydrate antigen 19-9 levels are elevated in up to 35% of patients with endometrial cancer and can be used in

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a follow-up evaluation of patients with mucinous borderline ovarian tumors.8, 9 In tumor marker tests CEA, AFP, CA27-29, CA19-9, and CA125 level are elevated but not more than 1000 units per ml in gastric carcinoma. 9 CA19-9 antibody is a monoclonal raised against colon carcinoma cells. The carbohydrate antigen is a glucoprotein; it is shown to react against a monosialoganglioside antigen. In colon cancer patients an elevated values of four folds, also increase in death compared to patients with lower values ($p< 0.001$). 10 Enzyme-linked immunosorbent assay ELISA is a very useful technique for the specific and sensitive assay of certain compounds, in which suitable antibodies, monoclonal or polyclonal, to the compounds are available. 11

Patients and methods:

Patients

The patients included in this study with age ranged 17-75 years, were classified into three groups as follows:

The first group included 8 patients with stomach cancer (G1). , The second group included 8 patients with colon cancer (G2). , The third group included 8 patients with rectum cancer (G3). ,The last group included 30 healthy subjects considered as control group (G4).

The patients were selected, during the period November 2004 to July 2005, according to the investigation of histopathologist. They were admitted for treatment at Medical City Hospitals (Baghdad Teaching Hospital and Nursing Home Hospital) and all surgical operations for all patients were carried out under the supervision of surgeons.

Preparation of Blood Samples

Five milliliters (mls) of venous blood were drawn from each patient by veinpuncture just before surgery, left to clot, and then centrifuged at 4000 r.p.m. for 30 min. Serum was separated and stored at -20˚C until time of analysis.

Collection of Specimens

The tumor tissue was surgically removed from patients. The specimens were immediately kept in normal saline solution and stored at -20˚C until the time of homogenizing process.

Homogenization of Tumor Tissues

The frozen tissue was sliced finely scalped in Petridish standing on ice, and then homogenized with three fold volumes of phosphate buffer pH7.4 by the homogenizer. The homogenate was filtered through nylon gauze to eliminate fiber connective tissues. The filtrate was centrifuged at 4000 r.p.m for 30 min at 4˚C in order to precipitate the remaining intact cells and the intact nucleus. The supernatant and precipitate fraction were separated and frozen at -20˚C until use.

Determination of (CA19-9) Antigen Using ELISA Assay

A basic ELISA work follows simple steps according to included manufacturer's instructions of CA19-9 Kit.

Results and discussion:

CA19-9 assay measures a tumor related antibody which is produced by adenocarcinoma of pancreas, stomach, gallbladder, colon, ovary and lung, and it is shed into the circulation. 12

The values for serum CA19-9 of all patients groups and control were shown in Table (1) and Figure (1).

CA19-9 in sera of G1, G2, G3 and G4 were (42.625± 33.088), (47.013 ± 0.318), (10.938 ±0.979) and (7.74 ± 4.915) U/ml respectively. The normal healthy subject has an upper limit of normal serum CA19-9 defined by a cutoff value of 37.0 U/ml.1, 2 The values for CA19-9 of our control group were within normal values with a range of (1-19) U/ml.

Table 1. CA19-9 value in serum in all patients groups

Patients Groups	No.	Mean± SD U/ml	Range U/ml	Under cutoff value %	Over Cutoff Values %
G1 (stomach)	8	42.625 ±33.088*	10-76	50	50
G2 (colon)	8	47.013 ± 0.318*	46.5-47.5		
G3 (rectum)	8	10.938 ± 0.979*	10-12.5		
G4 (control)	30	7.74 ± 4.915*	1-19		

* Significant P value< 0.005

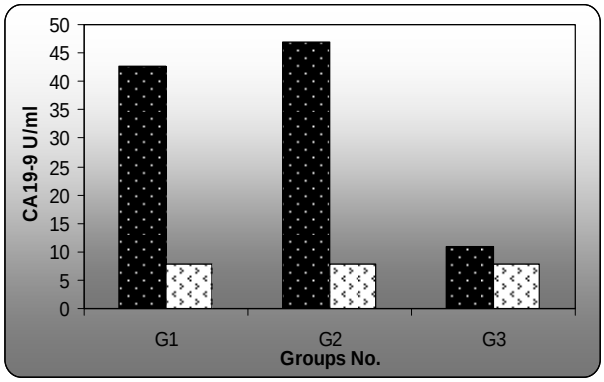

Figure 1 Serum CA19-9 levels of all patients groups and control

From the Table (1) and Figure (1) the values for CA19-9 in sera of stomach cancer patients G1 ranged (10-76) U/ml. About 50 percent of patients showed values of CA19-9 in their sera to be within the upper limit of the normal healthy subjects. Serum CA19-9 was not considered to have significance in the diagnosis of primary gastric carcinoma.13, 16. A study conducted on the

prognostic significance of gastric juice CA19-9 and sera CA19-9 in patients to diagnose gastric carcinoma showed that 42 of 69 patients had higher cutoff values of normal subjects. 17 CA19-9 in sera of colon cancer patients G2 showed a significant elevation than that of control. The values of CA19-9 in sera of rectum cancer patients G3 were within the cutoff value 37U/ml in authority 1, 2 but significant higher values were reported compared to the cutoff value 19U/ml, which was found in this study. Elevated serum levels of CA19-9 have been found in many patients with colorectal, gastric, biliary tract and pancreas carcinoma. 18, 19 CA19-9 and other tumor markers like CEA and CA72-4 are primarily used in early diagnosis and monitoring of therapeutics and determining the prognosis of patients who have undergone tumor resection. 20,21 To make comparative study between sera and tissues values, Table (2) and Figure (2) showed the results of CA19-9 in tumor tissue's homogenate of all groups studied.

Table 2. CA19-9 values in tissues homogenate in all patients groups

Patients groups	No.	Mean ± CD U/ml	Range U/ml
G1(stomach)	8	1170.25 ± 8.45 *	1160 - 1180
G2(colon)	8	535.75 ± 263.46 *	184 - 888
G3(rectum)	8	483.75 ± 16.37 *	448 - 492

* Significant P value< 0.005

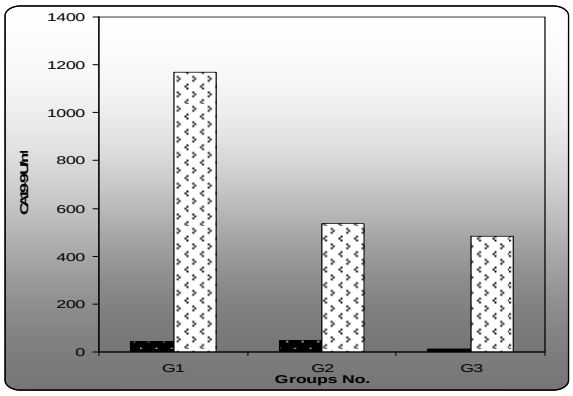

Figure 2. Tissue's homogenate CA19-9 and serum CA19-9 levels of all groups studied

The elevation in the carbohydrate antigen CA19-9 could be due to the increase in their expression and their ligand protein. A study conducted on the expression of some carbohydrate antigens and their roles in the invasion and metastasis of different cancer types (renal pelvis, ureter and urinary bladder), the results showed that there were differences between the organs depending on the expression of these antigens which frequently expressed in the tumor cells. Regardless of atypical grade, some of the antigen could be corrected with the stage and grade of tumor. Other antigens may also be involved in tumor invasion and metastasis.22 In conclusion, from the results above the values of CA19-9 in tissues are highest than values in the sera of the patients with the same type of cancer. This is an indicator that the cancer cells produce the antigen CA19-9 and release it to the blood. Moreover their high levels in tissue homogenates recommend further test to done cytological and histological to detect and diagnos these diseases.

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