

A Study of Carbohydrate Antigen 19-9 Level in Patients with Benign and Malignant Colorectal Tumors in Relation to the level of Immunoglobulins.

Samir M. Jasim¹PhD, Safa Al-deen Salim²PhD, Mehdi M. Reda³PhD.

Abstract

Background: CA19-9 has been regarded as an important tumor marker that is related to the presence of colon and rectal tumors.

Objective: To determine the concentration of serum CA19-9 and immunoglobulins in patients with benign and malignant colorectal tumors, in addition to the determination of the correlation coefficients between the concentrations of CA 19-9 and the different types of immunoglobulins.

Materials & Methods:

Patients: The subjects were 31 patients with malignant colorectal tumor, 31 patients with benign colorectal tumor and 31 volunteers healthy subjects.

Methods: The determination of the total concentrations of CA19-9 was carried out according to the procedure of IRMA, while the determination of the concentrations of immunoglobulins (IgA, IgM and IgG) was carried out using the radial immunodiffusion method RID.

Results: Results of the present study showed that the concentration of serum CA19-9 was

significantly elevated in patients with stages B, C and D (modified Duke classification) of colorectal carcinoma, while there was no significant variation in the concentrations of CA19-9 in sera of patients with benign lesion in comparison with control individuals. The results also showed that there was a significant correlation coefficient ($r = 0.875$) between the concentration of serum IgA and that of CA19-9 in patients with malignant tumors.

Conclusions: The humoral immunity, reflected by immunoglobulins was characterized by an increase in IgA level in serum of patients with colorectal carcinoma and this was concomitant with an increase in serum CA19-9.

Key words: Colorectal tumor, carbohydrate antigen 19-9, immunoglobulins.

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Introduction

Colorectal tumors are one of the most common tumors in human. However, there are tumors like lesions, which include the juvenile polyp, inflammatory polyp and hamartomatous polyp. They have the same presentation of tumors and may need the same line of treatment.

They can be differentiated only through histopathological study.

Tumor markers are substances normally produced in low quantities by cells in the body, when detected at levels more than normal by radioimmunoassay or immunohistochemical techniques usually indicates the presence of a certain type of cancer⁽¹⁾.

Carbohydrate Antigen 19-9 (CA-19-9) is a tumor marker for colorectal and many other tumors. The specificity of CA19-9 assay is about 99% for malignant gastrointestinal disease⁽²⁾.

Among the tumor-associated carbohydrate structures, which accumulate substantially in colorectal

¹Dept. Physiological Chemistry- College of Medicine- Al-Nahrain University,²Cancer Research unit. - College of Medicine - Kufa University,³Dept. Biochemistry - College of Medicine - Kufa University.

Address Correspondence to: Dr. Samir M. Jasim

E-mail: samir_jsm@yahoo.com.

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tumors, the blood group related sialyl Le^a and sialyl Le^x antigens, which are representative examples of type 1 and type 2 terminal structures respectively (3,4).

In short the epitope of sialyl Le^a antigen has a chemical structure isomeric to that of sialyl Le^x antigen (5). Neoplastic transformation is often associated with characteristic changes in the expression of the sialyl Le^a and sialyl Le^x antigens representing typical tumor associated carbohydrate antigens. High amounts of sialyl Le^a are present in human adenocarcinomas of the colon, pancreas and stomach (6). In adenocarcinomas of colon, stomach and pancreas, sialyl Le^a antigen was detected as monosialoganglioside (7).

The antigen is defined by a monoclonal antibody raised against determinants found in human colorectal cancer cells. The antigen is also found in normal fetal tissue as well as adult pancreatic fluid, gastric fluid, saliva and meconium. CA19-9 is removed from the circulation by the biliary system. The antigen is not expressed in persons with geno type Lewis (a-b-), which corresponds to about 5% of population (8).

Materials and Methods

i- Patients:

This study was conducted during the period May 2004 to April 2005, in the Hepatology and Gastroenterology – Teaching Hospital (Al- Najaf city), and Al-Kadhemia Teaching Hospital (Baghdad).

The subjects were 31 patients with malignant colorectal tumor group (age 25-75 yr.), 31 patients with benign colorectal group (age 22-70 yr.) and 31 blood samples obtained from volunteers, apparently healthy subjects (age 20-50 yr.) were selected as a control group. Subjects of all groups were evaluated by

full medical history under the supervision of a specialist physician, to exclude any existing systemic disease that may affect the parameters to be determined, particularly diabetes, liver disease, renal disease and chronic drug intake.

Samples were analyzed in the Dept. of Physiological Chem., College of Medicine, Al-Nahrain Univ.

ii- Determination of CA19-9 concentration:

A- Reagents:

- Monoclonal I¹²⁵-labeled anti CA19-9 monoclonal antibody in a vial contains 310 KBq with bovine albumin, sodium azide and a dye. (Immunotech-Beckman Coulter Company (Czech Republic)
- Six vials of standards contain the concentrations 0,15,30,60,120,480 U/ml of CA19-9.
- Anti CA19-9 monoclonal antibody coated tubes.
- One vial contains bovine serum albumin in buffer (Diluent).

B- Procedure:

The total concentration of CA19-9 was determined according to the procedure of the IRMA kit. Diluent (200 µl) was added to each of the antibody coated tubes. From each of the standard or sample a (50 µl) was also added. The tubes were then incubated with shaking (400 rpm) for 2 hrs. at room temperature (20-22 °C). The contents of the tubes were aspirated thoroughly and the tubes were washed with 2 ml of distilled water. Then (100 µl) of the tracer was then added to each tube and vortexed. Two tubes, each contains 100 µl of tracer were prepared separately for total radioactivity measurements after incubation for 1 hr. at (20-22 °C). All tubes were placed in Gamma Counter (Wallace 1470 Wizard) for one minute, in order to determine the radioactivity of

each tube. The amount of radioactivity for each tube refers to the amount of the bound CA19-9 to the inner surface of the

coated tube which can be represented by (B).

$$B/T \% = \frac{\text{Bound radioactivity of standard or sample C.P.M}}{\text{Total radioactivity C.P.M.}} \times 100$$

The standard curve was constructed by plotting B/T% against the corresponding concentration of CA19-9. From this curve the unknown concentrations of the samples were obtained.

iii-Determination of the concentration of immunoglobulins (IgA, IgM, IgG):

The concentrations of immunoglobulins were measured by radial immunodiffusion method (RID), in which the area circumscribed by the precipitation ring was proportionate to the antigen concentration.

Five µl of serum was taken for patients with benign and malignant colorectal tumors and dispensed into the well. The control samples were treated in the same way. After incubation at room temperature for 72 hr., the diameters of the immunoprecipitation rings for IgM, IgA and IgG were measured and compared with the reference values supplied by the manufacturer.

Results

Carbohydrate Antigen, CA19-9, concentrations were determined in sera of patients with benign, malignant tumors and control healthy individuals using immunoradiometric assay, IRMA.

Results in (Table 1) show that there is a significant increase in serum CA19-9 concentration in colorectal carcinoma patients, when compared with control group. However, there is no significant difference between serum CA19-9

concentration in benign tumor patients as compared with control group. The results of the present study showed also that there is no significant difference in CA19-9 concentration between male and female in all groups.

These results are in a good agreement with many earlier studies (9,10,11 and 12).

Results in (Table 2) summarise the values of serum CA19-9 concentration for patients with different stages of malignant tumors (A, B, C and D, according to the modified Duke's classification).

(Table 3) shows the correlation coefficient values of serum CA19-9 with serum IgM, IgA and IgG for patients with benign and malignant colorectal tumors. These results show that the correlation coefficient between IgA and serum CA19-9 in malignant colorectal tumor is positive ($r = 0.875$), ($p < 0.001$), while there was no correlation in benign tumor.

(Table 4) shows the mean values of serum IgM, IgA and IgG concentrations in patients with different stages of colorectal carcinoma and benign tumors, serum concentration of IgA was found to be increased in patients with stages B, C and D when compared with control.

Insignificant variation was observed in concentrations of IgM and IgG for patients with benign and

malignant colorectal tumors when compared with control individuals.

(Figure 1) shows the correlation representation of serum IgA with CA19-9 in malignant colorectal tumor.

Table 1: Serum CA19-9 levels in colorectal tumor patients and controls.

Groups	Number	CA19-9 U/ml	P-values
		Mean $\pm$ SD	
Colorectal carcinoma patient	31	108.24 $\pm$ 41.04	<0.001
Benign tumor patient	31	13.39 $\pm$ 5.08	N.S
Control	31	12.50 $\pm$ 3.95	

N.S: Not Significant.

Table 2: Serum CA19-9 levels in patients with different stages of colorectal carcinoma.

Stages of tumor	Number	CA19-9 U/ml	P-values
		Mean $\pm$ SD	
A	9	14.19 $\pm$ 3.57	N.S
B	14	75.56 $\pm$ 5.85	<0.01
C and D	8	127.32 $\pm$ 33.67	<0.001
Control	31	12.50 $\pm$ 3.95	

N.S: Not Significant.

Table 3: Correlation coefficients (r-values) of Serum CA19-9 with serum IgM, IgA, and IgG in patients with benign and malignant colorectal tumor.

Groups	r-value (CA19-9 with IgM)	r-value (CA19-9 with IgA)	r-value (CA19-9 with IgG)	P-values
Control	0.022	0.040	0.160	N.S
Benign tumor	0.132	0.210	-0.275	N.S
Colorectal carcinoma	-0.274	0.875	-0.303	<0.001*

N.S: Not Significant.

* : Significant between CA19-9 with IgA.

Table 4: Serum IgM, IgA and IgG concentration in patients with different stages of colorectal carcinoma.

Immuno - globulin s	Normal controls mean ± SD N=31	Rang values	Benign patients Mean ± SD n=31	Stage; A Mean ± SD n=9	Stage; B Mean ± SD n=14	Stage; C & D Mean ± SD n=8
IgM (mg/dl)	151.49 ± 48.51	40-250	144.82±41.22 (N.S)	165.63±20.51 (N.S)	153±42.84 (N.S)	114.27±5 1.99 (N.S)
IgA (mg/dl)	210.43 ± 54.52	90-310	198.23±45.77 (N.S)	224.63±39.51 (N.S)	321.74±91.54 (P<0.01)	380.57±1 07.67 (P<0.01)
IgG (mg/dl)	1082.57 ± 185.19	710-1520	1086.32±155.46 (N.S)	1105.18±165.42 (N.S)	1095.71±182.1 6 (N.S)	1041.6±2 05.40 (N.S)

n: number of cases

N.S: Not Significant.

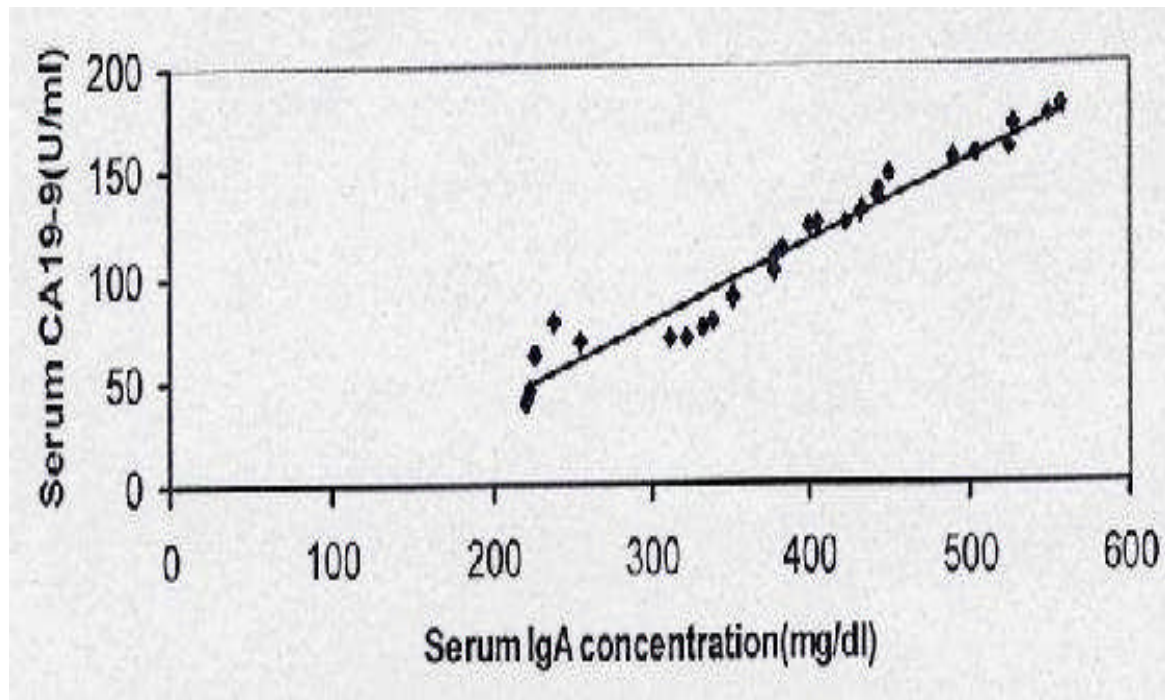


Figure 1: Correlation coefficients of serum CA19-9 with IgA in malignant colorectal tumor. ($r= 0.875$, $P<0.001$) $n=31$.

Discussion

Results in (table 1) show that there is a significant increase in serum CA19-9 concentration in colorectal carcinoma patients. Narimatsu (1998)⁽¹³⁾ showed that the increase in serum CA19-9 concentration is associated with a modification of the antigen expression. The synthesis of blood group antigens in carcinoma cells is believed to be as a consequence of the activation of specific glycosyltransferase, which is suppressed in normal cells. A gradual increase in the amount of tissue sialyl Le^a was found in colon and rectum during neoplastic transformation and progression.⁽¹⁴⁾

The increased amount of CA19-9 on the tumor cell surface can increase adhesiveness, which contribute to the formation of large tumor emboli. Metastatic spreads also facilitated by CA19-9 molecules, increasing the

adherence of tumor cells to the vascular endothelium of secondary sites of implantation and by increasing the ability to aggregate platelets.⁽¹⁵⁾

It can be seen from results in Table (2), that the mean values of the serum CA19-9 concentration in patients with stages (B,C) and (D) were significantly elevated, whereas, in patients with stage (A) there was no significant variation as compared with control group. These results indicate that CA19-9 concentration is significantly elevated in patients with metastatic disease and with increasing the degree of dysplasia or with the size of the lesion. During neoplastic transformation, the carbohydrate chains in glycolipids and glycoproteins are frequently altered. There is a close relationship between the expression of certain carbohydrate

antigens and oncogenesis. The significant elevation of tissue CA19-9 in stages (B,C) and (D) demonstrate the obvious relationship of the tumor marker in the infiltration phenomenon, that is associated with oncogenesis of colon and rectum⁽⁵⁾.

Both IgM and IgG in serum of patients with malignant and benign tumor have no correlation coefficient with serum CA19-9, however there is an increase in IgA level associated with the increase of CA19-9 level in colorectal carcinoma patients, (Table 3) and (Figure 1). This relation has been observed also by other workers⁽¹⁶⁾.

This correlation between CA19-9 and IgA is found in the different stages of malignant colorectal tumor (Table 4)) and can be considered as a parameter which may be beneficial in staging of these tumors and could be used also to predict the progression of colorectal carcinoma. These data might indicate the clinical utility of serum IgA as a potential complementary tumor marker to CA19-9 in the stages B, C, and D of colorectal carcinoma patients. Iarumov, (1998)⁽¹⁶⁾ suggested that serum IgA can be used also as complementary tumor marker to CA19-9, but in cases of relapses and metastasis.

Gurckoglu, (1998)⁽¹⁷⁾ have observed an increase in B type lymphocytes and a decrease in T cells in biopsy materials, they have found also a high level of IgA in the sera and duodenal liquid of colorectal carcinoma patients. These findings suggest to them that there is a role of cellular and humoral immunological alteration in the development of colorectal cancer.

IgA is the most important component in mucosal specific immunity. It is transported by Secretary

component (SC) on mucosal epithelial cell after production by cells or plasma cells in the lamina propria (LP)⁽¹⁸⁾.

These findings suggest a mechanism by which the number of IgA- containing plasma cells was closely related to (SC) staining of neoplastic mucosa and that (SC) may be important in the mechanism by which IgA is produced by lymphocytes to the lamina propria of the colon⁽¹⁹⁾.

Conclusions

A high level of serum CA19-9 was detected in colorectal carcinoma patients and this high level was observed with more advanced stages B, C and D. This parameter may be used as a prognostic indicator to predict the aggressiveness of the malignant tumor in colorectal carcinoma.

In addition to that the humoral immunity, reflected by immunoglobulins was characterized by an increase in IgA level in serum of patients with colorectal carcinoma and this was concomitant with the increased level of serum CA19-9.

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