CEA, CA19-9, & A-FP as Tumor Markers for Colorectal Carcinoma

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Summary:

Background: *Measurement of CEA, CA19-9, and A-FP are tumor markers for colorectal carcinoma, to be used for follow up and to detect early relapse.*
Setting: *specialized Surgical Baghdad -Teaching Hospital.*
Aim: *to shade a light on the sensitivity and specificity of these tumor markers (CEA , CA 19-9 , and a IT) for colorectal carcinoma .*
Patients and Methods: *A total of 30 patients with colorectal cancer were studied between June 2003 and April 2004, only 25 were followed up because the remaining 5 were beyond treatment as their CKA level was above 60 ng/ml. so they were excluded . The other patients were studied pie and 3 months post operative!}' as well 20 other non malignant G.I patients and 30 healthy controls . The serum was estimated for CBA , CA19-9 , and A-FP by RLFA (Enzyme Linked fluorescent Assay) method .*
Results: *The results show that there is significant difference between scrum level of CCA in pie- operative colorectal cancer patients and control ($P<0.0005$), and significant difference between the same patients and the post operative group ($P<0.05$) . While Tor the determination of serum CA 19-9 there arc insignificant differences between the pre-operative group and control, and between the pre- and post- operative groups ($P>0.05$) . As for the results for a FP There is significant difference between the pre-operative group patients and the non malignant Ci.I patients ($P <0.05$) , and significant difference between the pre- and post- operative groups ($P< 0.1$).*
Conclusion: *Significant differences were found in the result of tumor markers (I A , and a FP studied in patients with colorectal cancer as compared with the other non malignant (I.I patients and control , these results confirm that these markers are good predictors for colorectal cancer. While for CA 19-9 can only be useful in metastasis GIT carcinomas . The sensitivity of using the above mentioned tumor markers together is 84% , while the specificity is 66%.*
Keywords: *colorectal cancer, CEA, CA19-9 , & A-FP.*

Introduction:

Colorectal cancer is cancer of colon or rectum , it is a leading cause of cancer death in the United states and other industrial countries . It caused about 56.500 deaths in 1999. Many of these deaths happened because the cancer were found too late to be cured. If colorectal cancer is found early enough, it can usually be cured by surgery | I . 2 , 3 , 4] .

There seem to be un inconsistent relationship with respect to fat and sugar consumption, to scrum cholesterol, and to serum beta-lipoprotein with colorectal cancer. It seems that individuals in whom colorectal cancer developed shared the same level of serum cholesterol as the general population initially.{5}

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Bacteria are thought to play a role in the causation of colorectal cancer, presumably it is their action on ingested fat or metabolism that is the critical factor. [5].

Most if not all colorectal cancers arise from an adenoma. The risk of malignant transformation in any individual polyp is low. It increases with increasing size of the adenoma, and villous.

Adenomas are much more prone to malignant transformation than tubular adenoma. [6 .7.8].

Colorectal tumorigenesis is considered to be a multistep process, including hyper proliferative mucosa , adenomas , and carcinomas .

A combination of both environmental and genetic factors play an important part in pathogenesis . [8].

Tumor markers are endogenous substances produced by cancer cells and sometimes normal cells . They can be found in large amounts in the blood or urine of some patients with cancer . [9].

CEA is a glycoprotein consisting of ~ 60 % carbohydrate and a molecular mass of - 186 200 kDa. CEA exhibits considerable

heterogeneity, which appears In be attributable to variation in its carbohydrate side chain . Most of the carbohydrate is composed of mannose, galactose , JV- acetylglucosamine , fructose , and sialic acid . {10,11}

CEA is attached to the cell membrane by a glycosyl phosphatidyl inositol anchor and probably is released as a soluble form by a phospholipase C or phospholipasc D .[10. 11]

Its physiological function is still unknown , but there is a suggestion that CliA may play a role in protecting the colon from microbial infection possibly by binding and trapping infectious microorganisms .| 10 |

It was found that CEA was elevated in variety of cancers such as colorectal (70 %) , pancreatic.(55 %), gastric (50 %), lung (45 %), breast and uterine (40 %) .{12}

In general CKA is increased in most types of advanced adenocarcinomas as well as multiple hrui'Mi disorders.{10}

An elevated CEA before surgery may indicate a poorer prognosis . if it is high before surgery , the CEA should return to normal levels in 4-6 weeks if the cancer has been entirely removed . Levels above 5 U/ml. are considered abnormal .] 9 |

CA 19-9 is a glycoprotein with a molecular weight of 210,000 Da , that has unknown physiological function .{13}

It is synthesized by normal human pancreatic and biliary ductular cells and by gastric, colonic , endometrial, and salivary epithelia .[14 .151

Although the CA 19-9 test was first developed for use in detecting colorectal cancer, it is more sensitive to pancreatic cancer. If the CAI9-9 blood level is high in newly diagnosed patients this is usually means the disease is advanced. Abnormal levels are above 37 U/ml. {9}

a FP is one of certain proteins produced by the embryonic cells , normally produced by the foetal liver. the elevated plasma level of a FP in an adult indicates the presence of carcinoma. of the liver (Hepatocellular carcinoma). It is also raised in cancers of testis, stomach , pancreas , lung, ovary , and for detection of neural tube defect in pregnancy .

Level of a FP is decreased after surgical resection of liver cancer or treatment of germ cell tumor .{15}

Normal level of a FP are less than 20 ng/ml. the level increased with the size of the tumor. In small tumors , levels may be less than 20 . a FP is also elevated in acute and chronic hepatitis but seldom above 100 ng/ml .|9J

Material and Methods :

Blood samples were collected from 30 patients with colorectal cancer and they were followed up after 3 months of treatment, 5 of them were beyond treatment. their CEA results were above 60 ng/ml so their results were excluded .

The results of the remaining 25 were evaluated pre and 3 months post operatively similarly, blood samples were obtained from 20 patients with non malignant G.I conditions.

The non malignant G.I conditions were chronic cholecystitis, duodenal ulcer, gastric ulcer. cholangitis , diverticulilis and chronic gastritis .

Normal blood samples were obtained from 30 apparently healthy blood donor persons who were considered as a control.

All patients were attending Specialized Surgical Baghdad-Teaching Hospital between June 2003 and April 2004.

The samples were estimated for CEA , CA 19-9 , and a FP .The method used for estimation was ELFA by kits supplied by bioMerieux in the Central Health Lab.

Principle :

The KLFA (Ln/yme Linked Fluorescent Assay) method .

The assay principle combines a 2- step enzyme immunoassay sandwich method with a final fluorescent detection(ELFA).

The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. It is coated with anti- CEA monoclonal immunoglobulins {mouse}.The other reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

The sample is collected by the SPR then transferred into the well containing the conjugate - alkaline phosphate labeled polyclonal immunoglobulin (goat).

The sample/ conjugate mixture is cycled in and out of the SPR to speed up the reaction . Unbound conjugate is removed by washing . During the final detection step the substrate {4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample .At the end of the assay , results are automatically calculated by the instrument in relation to the calibration curve stored in memory , and then printed out .[16 |

Results :

A total of 80 individuals were studied , 30 apparently normal blood donor individuals used as controls, 20 patients with non malignant G.I diseases, and 30 patients with colorectal

carcinoma , 5 of them were beyond treatment so they were excluded , the remaining 25 were studied pre operative and followed 3 months post operative .

The mean age of the subjects ± **SEM** were 53.56 ± 2.41 , 53 ± 2.76 , 62.2 ± 2.95 years respectively , sec table I

Determination of scrum CEA in pro-operative colorectal cancer patients as compared with non malignant G.I. diseases patients , and controls was shown in Fig. 1 and table II .

The results indicate that all the patients have serum C E A value above 5 ng/ml , but insignificant difference between the non malignant G.I group and (he control group .While there is a significant difference between the colorectal carcinoma group and the control { P< 0.0005).

While the results of the patients post - operatively when compared with the same patient's pre - operatively are shown in Fig. II and table III.

The results show that there are significant differences between the prc-operalive colorectal cancer patients group and post-operative patients group (P < 0.05).

The results of determination of scrum CA19-9 in control, non malignant G.I. diseases patients group, and pre- operative colorectal cancer patients group are shown in Fig. III and table II.

The results shows that there is insignificant difference between the non malignant G.I. diseases patients group and control group { P>0.05).

And the results between the pre operative colorectal cancer patients group and control arc insignificant differences (P > 0.05).

And when the results of pre operative colorectal cancer patients group arc compared with the post operative group of the same patients , the results are shown in Fig IV and table III -

The results show insignificant differences between the posts - operative colorectal cancer patients group as compared with the same patient's pre - operatively (P> 0.05).

The mean levels of serum a FP between controls, non malignant G.I. diseases patients , and pre -operative colorectal cancer patients are shown in Fig V and table II. .

The results show that there is significant differences in the mean level of a FP between non malignant G.I. diseases patients group and control group (P< 0.05) , and there is a significant differences between the pre — operative colorectal cancer patients group and control group (P < 0.05).

As for the determination of a FP in pre - and post -operative colorectal cancer patients , the results are shown in Fig.VI and table III .

The results show that there is significant differences between the post-operative colorectal cancer patients as compared with the same patients pre-operatively (P< O.1)

When the sensitivity and specificity were studied between the 3 tumor markers mentioned above , the results are shown in table IV , the over all sensitivity was 84% and the specificity was 66%.

Discussion :

CEA was found to be elevated in a variety of patients with carcinomas such as lung , breast and GIT , as well as varies types of non malignant conditions including liver disease of varies types, pancreatitis. And inflammatory bowel disease {15,17}.

The cut off value of CEA was 5.5 ng/ml which was identical to that reported by many studies .{15} . The sensitivity was 40% while the specificity was 80% .

Many authors reported that the CEA level correlated well with tumor load {18}, and in this study. it can be observed that the levels of CEA in scrum may reflect the extent of tumor metastasis , therefore , the higher the value , the greater the extent of tumor volume .

So we can consider CEA as an independent prognostic factor in non - metastasis colorectal cancel" patients after curative surgery. [19]

But it should be noted that the elevation occur only in patients with advanced disease , not all patients with recurrent colorectal cancer will exhibit increased level , high level may occur in conditions unrelated to recurrent colorectal cancer. and certain catatonic therapies may cause transient increased concentrations .

Serum elevation was detected in the specimens from different G.I. cancers or from normal pancreas , stomach , liver, and gall bladder. The serum concentration were elevated particularly in patients with pancreatic cancer but also in patients with other G.I cancers or some benign G.I diseases { 13].

The reported cut oft value of normal scrum CA 19-9 was less than 37.3 U/ml which is more or less similar to these obtained by earlier reports .

The calculated sensitivity of this study was 70% while the specificity was 65% and it is also more or less similar to other studies.

However , most studies suggest that the CA 19-9 had low sensitivity for colorectal cancer and appear lo be more sensitive for gastric , biliary tract, and pancreatic carcinomas | 17 |.

It seems dial CA 19-9 level is one of the best available prognostic indicators in advanced colorectal carcinoma . {19}

In the present study of a FP , the upper limit was < 1.9 ng/ml and the specificity 50 % while the sensitivity was 70% . it seems that a FP is more

predictable in liver carcinoma (hepatocellular carcinoma) and other malignant diseases that has liver origin .

Conclusion :

Significant differences were found in the result of tumor markers CEA and a FP patients studied in patients with colorectal cancer as compared with the other non malignant G.I patients and control, these results confirm that these markers are good predictors for colorectal! Cancer. While for CA 19-9 can only be used in metastasis GIT carcinomas . The sensitivity of using the above tumor markers together is 84%, while specificity is 66%.

Table I : Age and gender in normal healthy individuals , patients with non malignant G.I diseases , and patients with colorectal carcinoma .

Individual characteristics	Control	Non malignant G.I. diseases	Colorectal carcinoma
Age (X±SEM) years	53.56 ± 2.41	53 ± 2.76	62.2 ± 2.95
Gender			
Male	19	13	19
Female	11	7	21
Sex ratio(male:female)	1.7 : 1	1.8 : 1	1 : 1.1

Table II : Determination of serum CEA , CA 19-9 , and α FP in colorectal carcinoma patients , patients with non malignant G.I. disease , and control .

Study Groups	No. of individual	Mean	± SEM	P t-test
		Serum level of CEA (ng/ml)		
Controls	30	2.62	0.26	
Non malignant G.I patients	20	2.66	0.43	*> 0.05*
Pre-operative colorectal cancer patients	25	16.06	0.693	*< 0.0005*
		Serum level of CA 19-9 (U/ml)		
Controls	30	25.19	1.23	
Non malignant G.I patients	20	40.39	8.05	*> 0.05*
Pre-operative colorectal cancer patients	25	14.85	0.425	*> 0.05*
		Serum level of α FP (ng/ml)		
Controls	30	0.8	0.3	
Non malignant G.I patients	20	1.8	0.6	*< 0.05*
Pre-operative colorectal cancer patients	*25*	*1.73*	*0.138*	*< 0.05*

Table III : Determination of serum level of CEA , CA 19-9 , and α FP in pre- and post- operative cancer patients .

Groups	No. of patients	Mean	± SEM	P t-test
		Serum level of CEA (ng/ml)		
Pre-op. colorectal ca.	25	16.06	0.693	
Post-op. colorectal ca.	25	3.587	0.370	*< 0.05*
		Serum level of CA 19-9 (U/ml)		
Pre-op. colorectal ca.	25	14.85	0.425	
Post-op. colorectal ca.	25	0.997	0.540	*> 0.05*
		Serum level of α FP (ng/ml)		
Pre-op. colorectal ca.	25	1.73	0.138	
Post-op. colorectal ca.	*25*	*1.207*	*0.120*	*< 0.1*

Table IV : The specificity and sensitivity of CEA , CA 19-9 , and α FP in colorectal carcinoma patients .

Tumor Marker	Sensitivity	Specificity
CEA	80%	*80%*
CA 19-9	70%	*65%*
α FP	70%	*50%*
CEA + α FP	85%	*70%*
CA 19-9 + α FP	70%	*50%*
CEA + CA 19-9	88%	*80%*
CEA + CA 19-9 + α FP	9[illegible]%	*66%*

Fig I : Mean level of serum CEA in control , non malignant G.I patients ,and pre-operative colorectal cancer patients.

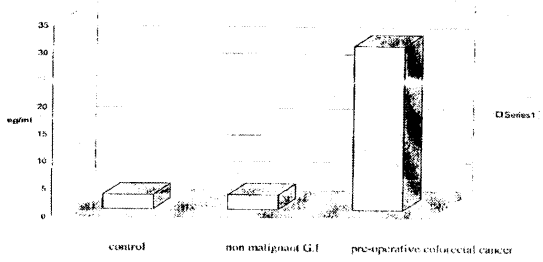


Fig II : Mean level of serum CEA pre- and post- operatively in patients with colorectal cancer.

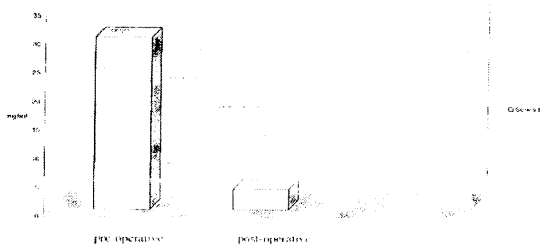


Fig III : serum level of CA 19-9 of controls , non malignant G.I diseases patients , and pre- operative colorectal cancer patients .

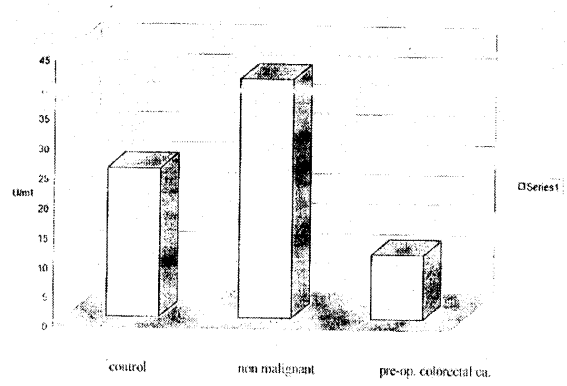


Fig IV : Serum level of CA 19-9 in pre - and post - operative colorectal cancer patients .

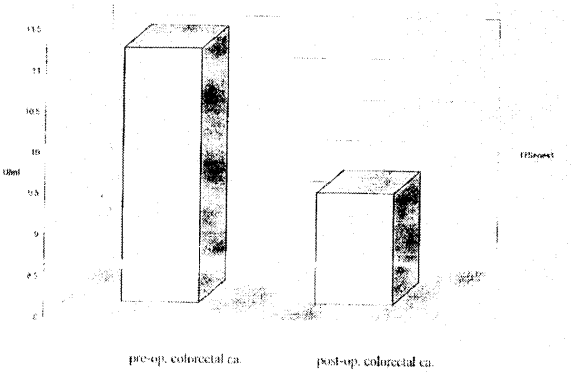


Fig V : Mean level of serum α FP in controls , non malignant G.I. diseases patients , and pre –operative colorectal cancer patients .

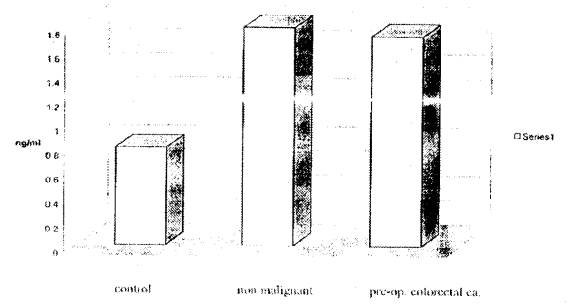
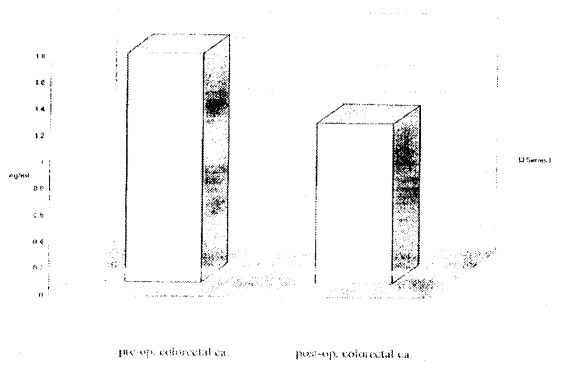


Fig VI : Mean level of serum α FP in pre- and post- operative colorectal cancer patients .



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