ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Comparative study of Carcinoembryonic antigen and Carbohydrate antigen 724 in Sera and Tissue of patients of Colorectal tumor

[1]Mohammed E. AL-Ghurabi *, [2]Rihab H. AL-Mudhafer. [3]Hamid K. AL-tameemi

[1]Department of Biology, Faculty of Sciences, University of Kufa. [2]Department of Pathology, Faculty of Medicine, University of Kufa. [3]M. Sc. student, Department of Biology, faculty of Science, University of Kufa, Najaf, Iraq

Abstract

Objective: the objectives of the study were to comparative measurement of CEA and TAG 72 in sera and tissue of patients with colorectal tumors and determining whether serum biomarkers are diagnostic markers or not.

Methods: 105 cases were included in this study divided into 3 groups of patients, patients with colorectal carcinoma, patients with adenomatous polyps(adenoma) and patients with non-specific colitis, 57 male and 48 female, 20 male and 15 female of carcinoma cases. Age range 42-79 years with mean 60.5 years. Paraffin blocks and blood samples were collected from these three group.

Results: TAG 72 was shown more specificity and sensitivity than CEA marker in diagnosis colorectal carcinoma and adenomatous polyps, their concentration in colorectal carcinoma more than adenomatous polyps. CEA and TAG 72 were given positive as percentages for poorly differentiation carcinoma more than moderated and well differentiated. **Conclusion:** Biomarkers may aid in differentiating benign from malignant carcinoma. Measurement of the level of biomarkers in the serum is better than the tissue in the detection and differentiation between types of tumors. Serum biomarkers are reliable markers in detecting colorectal carcinoma. Complementary use of CEA and TAG 72 give more accurate results.

Key words: CEA, TAG 72, colorectal carcinoma, adenomatous polyps, CA 724, Colitis, specificity and sensitivity**.**

INTRODUCTION

Colorectal carcinoma is common malignancy in Europe and America, but low in Africa and Asia. In the US colorectal carcinoma is the third most common cause of cancer and second leading cause of death, at the same time it is the most curable cancer among GIT malignancies [1].

The colorectal carcinoma can result from many causes, including both genetic and environmental factors. Both of these causes finally lead to mutation(s) in the DNA [2]. Many of molecular changes on genome level that in the end cause the cell to lose their ability to control division . Many mutations associated with transformation of normal cell to neoplastic cells, like mutations occuring in tumor suppression genes(that normally repair any damage that occur in the DNA i.e. suppress the mutations) and oncogenes [3]. Colorectal carcinoma has many markers that appear in the tissue and serum, and these biochemical markers have very significant role in testing the early stages of disease, helping in diagnosis, monitoring the patient undergoing the resection and in checking the treatment in advanced cases [4].

Carcinoembryonic antigen(CEA) is one of the most common tumor markers largely used in colorectal carcinoma. It was characterized at the first time in (1965) by the Scientists Gold and Freedman[5]. CEA levels in serum and tissues elevated with advanced tumor stage, its diagnosis in 50% of patients with tumor spread to lymph nodes and the percentage arise to 75% in patients with tumor metastasis; therefore, it is very significant in the diagnosis [6].

The second most significant marker described in colorectal carcinoma is carbohydrate antigen 72-4 [7]. Also called Tumor Associated Antigen 72(TAG 72) is large glycoprotein associated with malignant cells in their cell membrane and cytoplasm. CA 72.4 is considered most important of colorectal markers due to its high sensitivity for colorectal carcinoma because it is expressed in about 80% of cases, in early stages of carcinoma and are not expressed in normal tissues or other benign disease [8].

MATERIALS AND METHODS

The present study achieved in laboratory of advanced researches of biology department in faculty of science and histopathology department in faculty of medicine /Kufa University. During the period from September 2016 and to February 2017, Written informed consent was obtained from all participants included in this study and the scientific ethical committee approved the project.

Inclusion and exclusion criteria

Only Iraqi patients, diagnosed with colorectal carcinoma during the period from 01 September 2016 through 31 February 2017, verified by histopathology report were included in the study, Non-Iraqi nationals, those iraqi who were diagnosed before 01 September 2016 or after 31 February 2017, smokers, patients with non-neoplastic polyps and cases without histological report were all excluded from the study.

Study groups

A hundred and five cases were included in this study divided into 3 groups of patients, patients with colorectal carcinoma, patients with adenomatous polyps(adenoma) and patients with non-specific colitis, 57 male and 48 female, 20 male and 15 female of carcinoma cases. Age range 42-79 years with mean 60.5 years. Paraffin blocks and blood samples were collected from these three group.The samples were collected from various sources,

the samples were collected from GIT endoscopy center in AL-Sader medical city in AL-Najaf province, AL-Kafeel hospital in Karbala province, AL-Gawadeen medical city and Baghdad medical city in Baghdad province, in Iraq.One-hundred-five paraffin blocks were sectioned and stained for CEA and CA 72.4. paraffin block of breast carcinoma were sectioned and stained as positive control for CEA with each run , And paraffin block of breast carcinoma was sectioned and stained as positive control slides for TAG 72 with each run.

ELISA Methods

Estimation CEA marker, Elabscience company(code E-EL-H0613). Estimation CA7.24marker,Elabscience company(code E-EL-HO643).

Immunohistochemistry methods

Estimation CEA marker, Bio SB/USA company.Estimation TAG 72 marker, Bio SB/USA company.

Statistical analyses

Statistical analyses were made with SPSS version 21 (SPSS Inc., Chicago, Illinois, USA), Chi-square test and Z test were used for comparing dichotomous variables and for testing differences in proportions between groups. The chi-square (goodness of fit) test was used to compare two nonparametric groups, the ROC curve was performed to reveal proportions of Sensitivity and Specificity of biomarkers, A P-value <0.05 was considered as statistically significant.

RESULTS

RESULTS OF SERUM

Concentration of CEA in patients with colorectal carcinoma, adenomatous polyps and colitis.

CEA concentration was statically significantly different in three patients groups. CEA concentration in colorectal carcinoma patients group was highly increasing(p<0. 01) as compared with colitis group. The mean, std. Deviation, T test and P value for CEA in colorectal carcinoma patients group were (7.016, ±2.583, 18.62 and <0.0001 respectively) as shown in table(4-1). There is significant increase(p<0.01) of CEA concentration in adenomatous polyp patients group as compared with colitis group). The mean, std. deviation, T test and P value for CEA in colorectal adenomatous polyp patients group were (2.036, ±1.205, 7.643 and <0.0001 respectively) as shown in table(4-1). Also there is highly significant increase(P<0.01) of CEA concentration in colorectal carcinoma patients group as compared with adenomatous polyp patients group, as shown in Graph (4-1).

Concentration of TAG 72 in colorectal carcinoma, adenomatous polyps and colitis patients group.

TAG 72 concentration was of statically significant difference in three patients group. TAG 72 concentration was of highly significant increase(p<0. 01) in colorectal carcinoma patients group as compared with colitis group as shown in figure(4-2), The mean, std. deviation, T test and P value of TAG 72 in colorectal carcinoma patients group were (19.71, ±4.694, 28.65 and <0.0001 respectively) as shown in table(4-2). There is significant increase in TAG 72 concentration in colorectal adenomatous polyp patients group as compared with colitis group as shown in figure(4-2). The mean, std. deviation, T test and P value of TAG 72 in colorectal adenomatous polyp patients group were(12.43, ±2.721, 28.20 and <0.0001 respectively) as shown in table(4-2). The concentration of TAG 72 in colorectal carcinoma patients group was more than in colorectal adenomatous patients group, as shown in Graph (4-2).

Serum CEA Sensitivity and .Specificity in colorectal Carcinoma patients group and adenomatous polyp patients group.

CEA sensitivity and specificity in carcinoma patient group were viewed in Graph(4-3). The sensitivity and specificity of CEA in carcinoma patient group were (77.36 % and 86.52%) respectively as shown in table(4-3). Area, std. Error, P value, were 0.9262, 0.02506, <0.0001, 77.36066% and 86. 52459% respectively as shown in table(4-3).

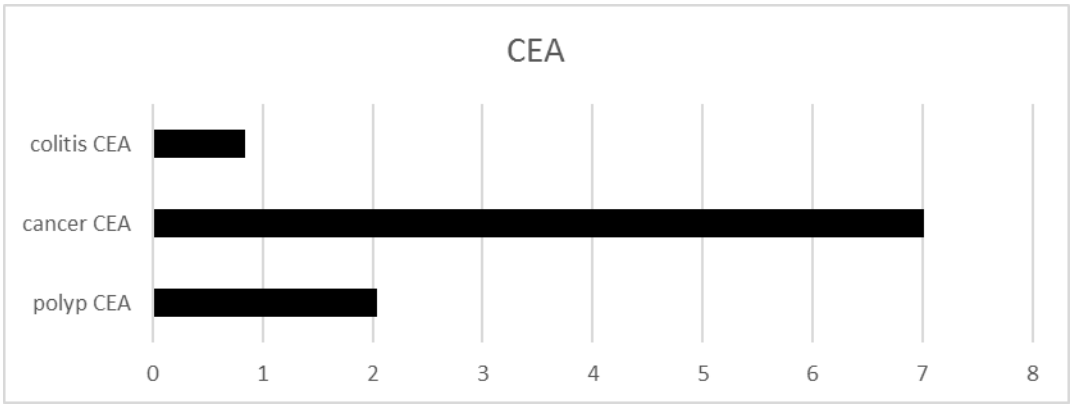
CEA. Sensitivity. and specificity. of adenomatous polyp patients group were viewed in Graph (4-4). The. sensitivity. and specificity. respectively were 67.36% and 77.52%. The area, std. Error, P value, sensitivity and specificity of CEA in colorectal adenomatous patients group were 0.8697, 0.03642, <0.0001, 67.36066 %and 77.52459% respectively as shown in table(4-4). The Graphs (4-3) and (4-4) show statically increase significance(<0.01) of sensitivity and specificity for CEA in colorectal carcinoma patients group as compared with colorectal adenomatous polyp patients group.

Sensitivity and specificity of serum TAG 72 in colorectal carcinoma patients group.

The sensitivity and specificity. of TAG 72 in carcinoma patients group are shown in Graph(4-5). The sensitivity and specificity were 88.52% and 100% respectively. Area, std. Error, P value, sensitivity and specificity of TAG 72 in colorectal carcinoma patients group were(0.9312, 0.02530, <0.0001, 88.52459% and 100%) respectively, as shown in table(4-6). The sensitivity and specificity of TAG 72 in colorectal adenomatous polyp patients group are shown in Graph (4-6). The sensitivity and specificity are(80.32% and 98.36%)respectively. Area, std. Error, P value, sensitivity and specificity of TAG 72 in colorectal carcinoma patients group were (0.9063, 0.02970, < 0.0001, 80.32787%and 98.36066%) respectively, shown in table(4-6). The Graphs (4-5) and (4-6) show statically increase significant(<0.01) of sensitivity and specificity for TAG 72 in colorectal carcinoma patients group as compared with colorectal adenomatous polyp patients group.

	colitis CEA	carcinoma CEA	polyp CEA
±Mean U/mL	0.8359	7.016	2.036
±Std. Deviation	±0.2262	±2.583	±1.205
Z test		18.62	7.643
P value		< 0.0001	< 0.0001

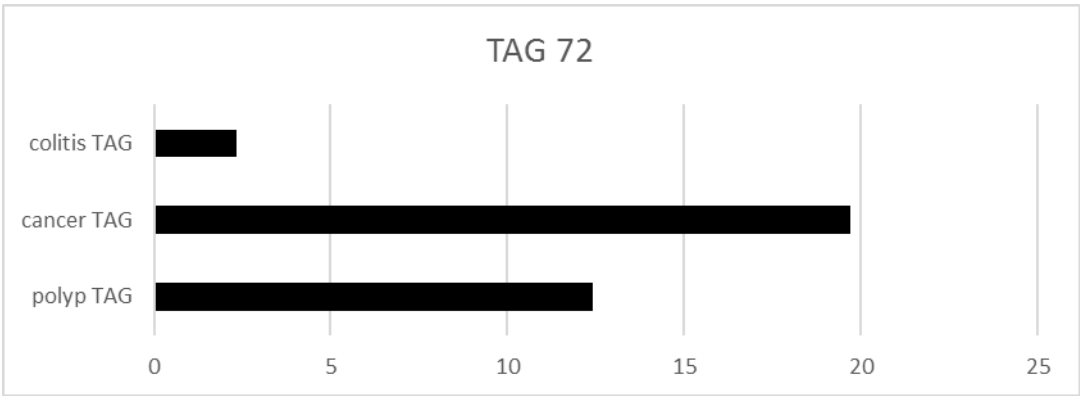
Table(4-1): shows the mean, std. Deviation, T test and P value in carcinoma CEA, polyp CEA and colitis CEA.



Graph(4-1): Concentration of the CEA in colorectal carcinoma ,adenomatous polyp and colitis patients group.

Table(4-2): Mean, std. deviation, T test and P value in carcinoma TAG 72, polyp TAG 72 and colitis TAG 72.

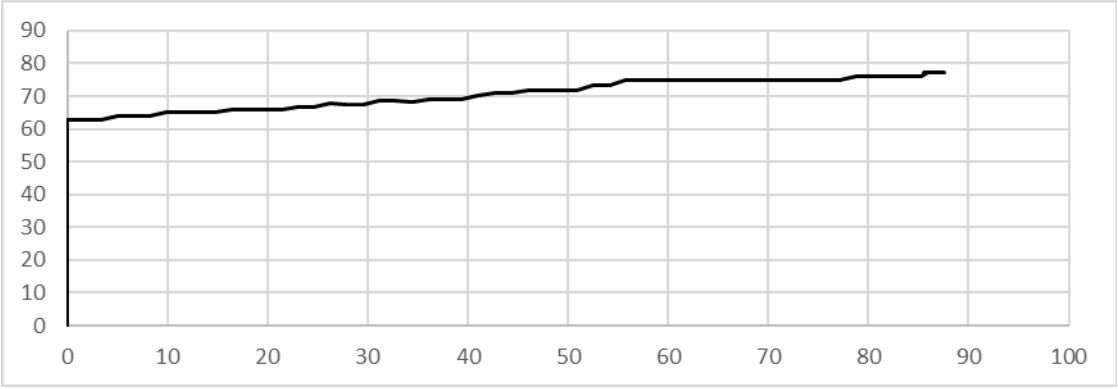
	colitis TAG 72	**carcinoma TAG 72**	**polyp TAG 72**
±Mean U/Ml	2.326	19.71	12.43
±Std. Deviation	±0.6533	±4.694	±2.721
Z test		28.65	28.20
P value		< 0.0001	< 0.0001



Graph(4-2): Concentration of the TAG 72 in colorectal carcinoma ,adenomatous polyp and colitis patients group.

Table(4-3): Area, std. Error, P value, sensitivity and specificity of CEA in colorectal carcinoma patients group.

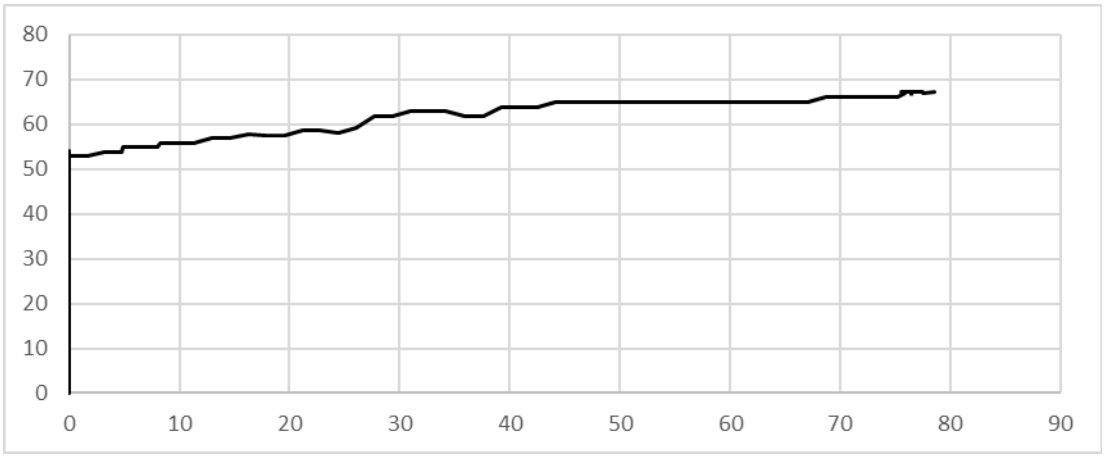
Marker	Area	Std. Error	P value	Sensitivity	Specificity
CEA	0.9262	0.02506	< 0.0001	77.36066	86. 52459



Graph(4-3):ROC curve view to CEA sensitivity and specificity of in colorectal carcinoma cases.

Table(4-4): Area, std. Error, P value, CEA sensitivity and CEA specificity of in adenomatous patients group

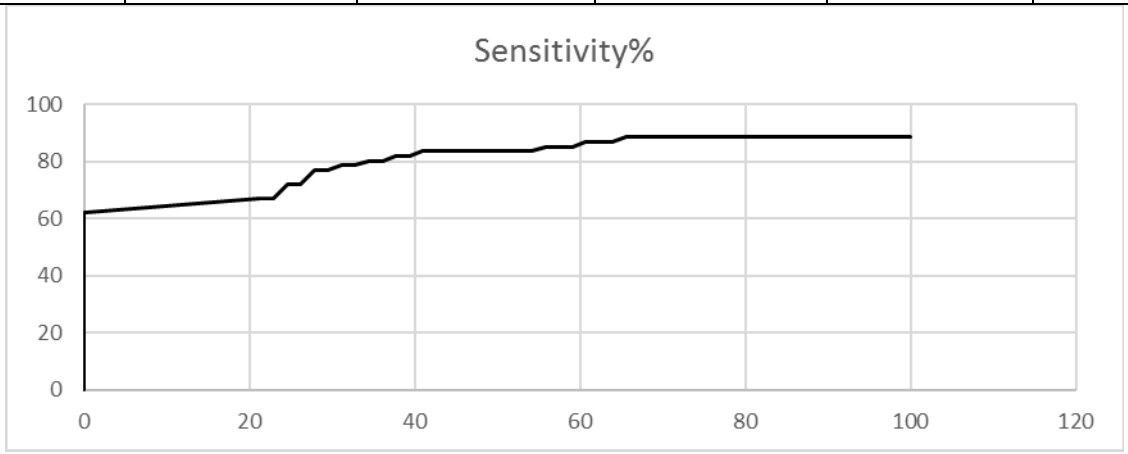
marker	Area	Std. Error	P value	Sensitivity	Specificity
CEA	0.8697	0.03642	< 0.0001	67.36066	77.52459



Graph(4-4):ROC curve view to sensitivity and specificity% of CEA in adenomatous polyp patients group.

Table(4-5): Area, std. Error, P value, sensitivity and specificity of TAG 72 in colorectal carcinoma patients group.

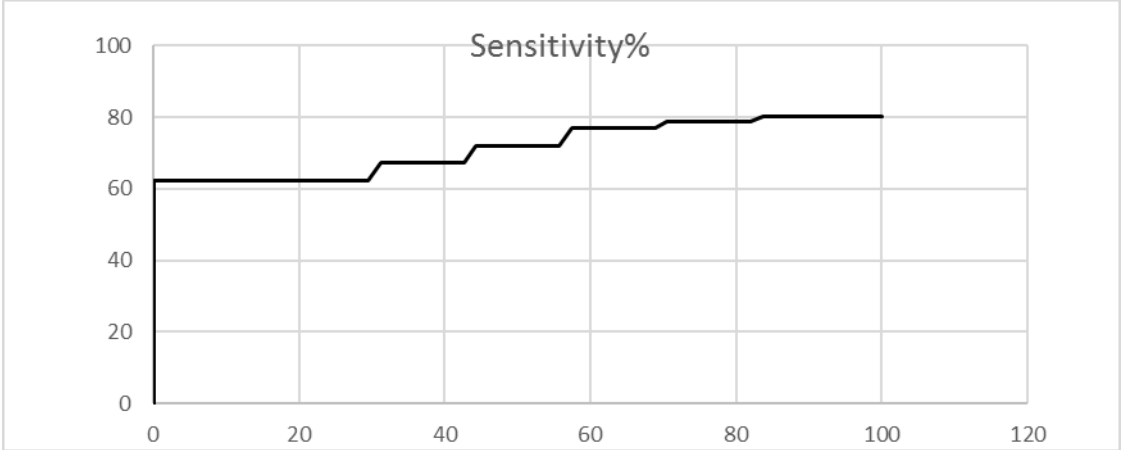
Marker	Area	Std. Error	P value	Sensitivity	Specificity
TAG 72	0.9312	0.02530	< 0.0001	88.52459	100



Graph(4-5):ROC curve view to sensitivity% and specificity% of TAG 72 in colorectal carcinoma patients group.

Table(4-6): Area, std. Error, P value, Sensitivity and specificity of TAG 72 in colorectal adenomatous patients group.

marker	Area	Std. Error	P value	Sensitivity	Specificity
TAG 72	0.9063	0.02970	< 0.0001	80.32787	98.36066



Graph(4-6):ROC curve view to sensitivity% and specificity% of TAG 72 in adenomatous polyp patients group.

TISSUE MARKERS

The sensitivity and specificity of CEA and TAG 72, and true positive and false positive in colorectal carcinoma patients group and colorectal adenomatous polyp patients group.

The sensitivity and specificity of both CEA and TAG 72 in both the colorectal carcinoma and colorectal adenomatous polyp patients groups shown in table(4-7), which also viewed the true positive ratio and false positive ratio to both. According to table (4-7) the sensitivity and specificity of TAG 72 in colorectal carcinoma patients groups were 80.00% and 94.29% respectively. And the true positive and false positive for same patients group were 93.33% and 82.25% respectively. Whereas the sensitivity and specificity of TAG 72 in colorectal adenomatous patients group were 54.29% and 94.29% and the true positive and false positive were 90.84% and 67.35% respectively.

The sensitivity and specificity of colorectal carcinoma patients group with CEA were 57.14% and 85.71% respectively. The true positive and false negative were 80.00% and 66.67% respectively as shown in table(4-7), while the sensitivity and specificity of TAG 72 in colorectal adenomatous patients group were 34.29% and 85.71% and the true positive and false positive were 70.59% and 56.60% respectively.

Comparative study of colorectal carcinoma and colorectal adenomatous polyp patients group with colitis patients group in negative and positive cases.

Table(4-8) shows the total numbers of colorectal carcinoma cases , colorectal adenomatous polyp and colitis cases which give positive or negative to CEA or TAG 72 .

Twenty eight cases of colorectal carcinoma out of 35 were positively stained for TAG 72 (80%). In the same group, 20 cases out of 35 were positively stained for CEA (57%) as shown in table(4-8).

Nineteen case of adenomatous polyp cases out of 35 were positively stained for TAG 72(54%). In the same group, 12 cases out of 35 were positively stained for CEA (34%) as shown in table(4-8).

Two cases of colitis cases out of 35 were positively stained for TAG 72(6%). In the same group, 5 cases out of 35 were positively stained for CEA (14%) as shown in table(4-8).

According to table(4-8) colorectal carcinoma cases positively stained for TAG 72 show statically significant increase when compared with CEA marker(x^2=24.242 and $p<0.05$), and also TAG 72 positive colorectal adenomatous polyp cases were more than CEA marker (x^2=2.837 and $p<0.05$). There are no significance different between colitis positive for TAG 72 and cases positive for CEA (x^2=1.429and $p<0.05$).

The grades of colorectal carcinoma cases for positive and negative CEA and TAG 72 markers

Eleven out of 16 well differentiated (grade I) colorectal carcinoma cases were positive for TAG 72 (68.75%),while 14 case out of 16 moderately differentiated carcinoma cases(grade II) were positive for TAG 72 (87.5%) and 3 out of 3 poorly differentiated cases were positive for TAG 72(100%) as shown in table(4-9).

Eight out of 13 well differentiated colorectal carcinoma (grade I) cases were positive for CEA (61.53%),while 10 case out of 19 moderately differentiated carcinoma cases(grade II) were positive for CEA (52.63%) and 2 out of 3 poorly differentiated cases were positive for CEA (66.66%) as shown in table(4-9).

By comparing the percentages of cases which give positive for both markers with grades show that the percentage increased from well to poorly differentiated as shown in table(4-9).

Complementary of both markers

In the current study, it was noticed that 3 out of 7 negative cases for TAG 72(42%) are positive for CEA, and 5 out 15 cases negative for CEA are positive for TAG 72(33%) suggesting a complementary results.

Table(4-7): view the sensitivity, specificity , true positive and false negative for both CEA and TAG 72 72.

Markers		Sensitivity%	Specificity%	+PV%	-PV%
TAG 72	Carcinoma	80.00	94.29	93.33	82.50
	Polyp	54.29	94.29	90.48	67.35
CEA	Carcinoma	57.14	85.71	80.00	66.67
	Polyp	34.29	85.71	70.59	56.60

Table(4-8): Shows the comparative study of colorectal carcinoma and colorectal adenomatous polyp patients with colitis patients groups.

n=35		Positive		Negative		total	X2 test	P value
		NO	%	NO.	%			
Carcinoma	TAG 72	28	80	7	20	35	4.242	0.0197
	CEA	20	57	15	43	35		
Polyp	TAG 72	19	54	16	46	35	2.837	0.0461
	CEA	12	34	23	66	35		
Colitis	TAG 72	2	6	33	94	35	1.429	0.1160
	CEA	5	14	30	86	35		

Table(4-9): Shows the grades of colorectal carcinoma cases for positive and negative CEA and TAG 72 markers.

n=35	TAG 72				CEA			
Histological tumor grade	Positive(n=28)		Negative(n=7)		Positive(n=20)		Negative(n=15)	
	NO.	%	NO.	%	NO.	%	NO.	%
Well differentiated	11	68.75%	5	31.25 %	8	61.53%	5	38.47%
Moderately differentiated	14	87.5%	2	12.5%	10	52.63%	9	47.37%
Poorly differentiated	3	100%	0	0%	2	66.66%	1	33.44%
	X2 test	10.94	X2 test	9.500	X2 test	10.67	X2 test	8.750
	P value	0.0042	P value	0.0087	P value	0.0048	P value	0.0126

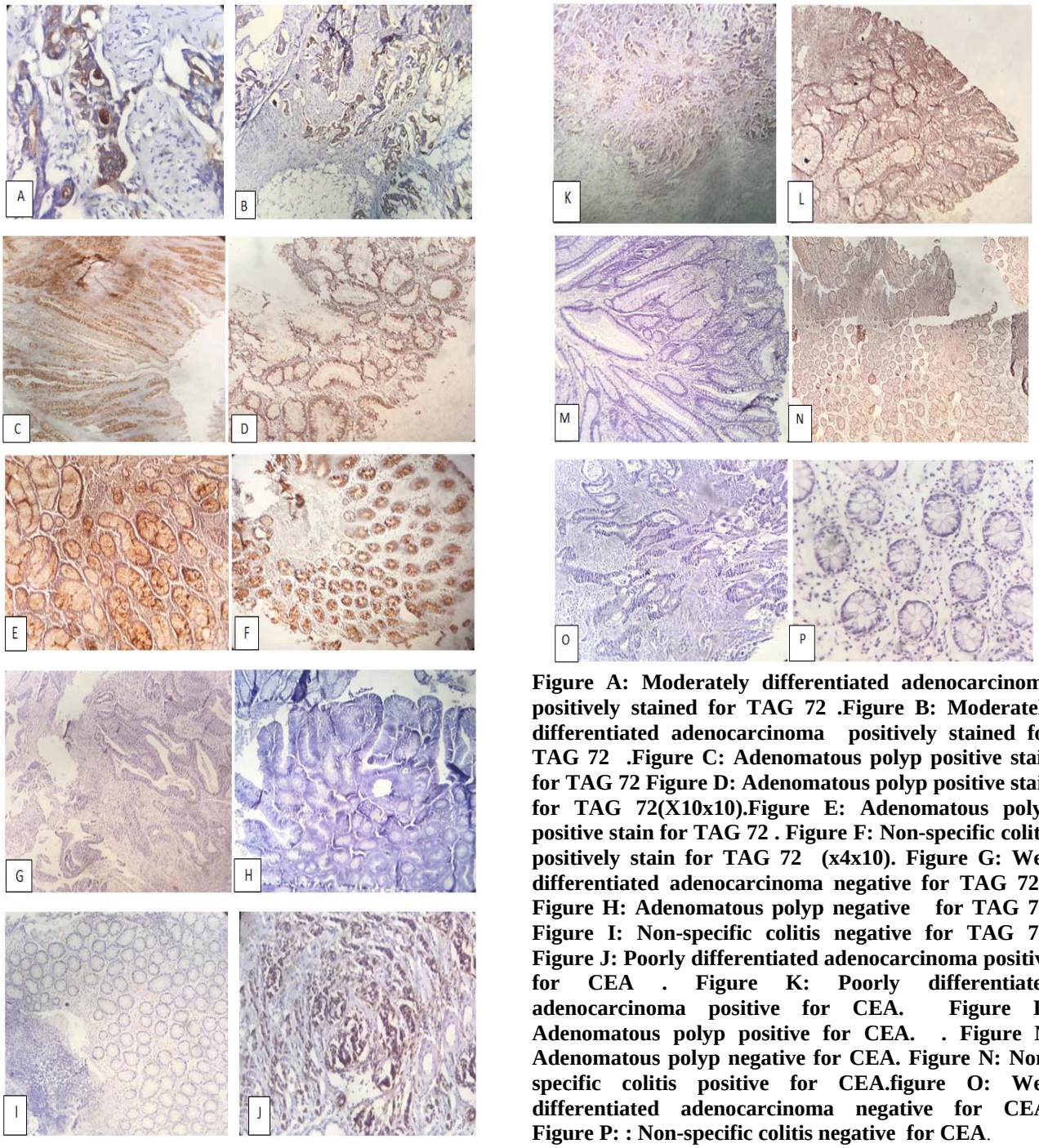


Figure A: Moderately differentiated adenocarcinoma positively stained for TAG 72 .Figure B: Moderately differentiated adenocarcinoma positively stained for TAG 72 .Figure C: Adenomatous polyp positive stain for TAG 72 Figure D: Adenomatous polyp positive stain for TAG 72(X10x10).Figure E: Adenomatous polyp positive stain for TAG 72 . Figure F: Non-specific colitis positively stain for TAG 72 (x4x10). Figure G: Well differentiated adenocarcinoma negative for TAG 72 . Figure H: Adenomatous polyp negative for TAG 72. Figure I: Non-specific colitis negative for TAG 72. Figure J: Poorly differentiated adenocarcinoma positive for CEA . Figure K: Poorly differentiated adenocarcinoma positive for CEA. Figure L: Adenomatous polyp positive for CEA. . Figure M Adenomatous polyp negative for CEA. Figure N: Non-specific colitis positive for CEA.figure O: Well differentiated adenocarcinoma negative for CEA. Figure P: : Non-specific colitis negative for CEA.

DISCUSSION

In our study, 105 cases had been taken and included 57 males and 48 females. Thirty five patients were diagnosed with colorectal cancer ,20 males and 15 females. Thirty five cases were diagnosed with adenomatous polyps and 35 cases were with colitis as control.

The results of this study explain there is no significant difference between the colorectal cancer and the gender. This result is in agreement with study done by (Jemal A et al., 2011)[9] which found that the prevalence rate of colorectal cancer is the same in both male and female. Also this result agrees with study done by Roy and Bianchi, (2009) and Zisman et al; (2006) [10, 11]. which suggest that the chances of colorectal cancer development are the same in both genders.

In the same time, this study disagrees with a study done by Ferlay et al(2013) [12]. , which found that the incidence in male is more than female. This contrast occur may be due to high number of cases included in his study, and also may be due to the female intake oral contraceptive(OCs) which reduce the risk level of colorectal cancer about 18% (Ricchi et al., 1999)[13].

The present study showed there is no significant difference between advance of age and development of the colorectal cancer. This result disagreement with result done in US by Edward BK et al., 2002[14]., which found the colorectal cancer has high prevalence in patients above 75 years. This contrast may be due to the present study took limited numbers of cases above 75 years.

Discussion of serum result.

Sensitivity and specificity of serum CEA in colorectal cancer patients group.

The sensitivity and specificity of CEA in colorectal cancer patients group is 77.36 % and 86.52% respectively as shown in figure (4-3).

Study done by Louhimo et al(2002) [15] has shown the sensitivity and specificity of CEA as 44% and 87% respectively. Carpelan-Holmstrom et al (1996) [16],explained that the sensitivity and specificity was 43% and 87% respectively. Hashem et al(2007) [17] conducted study and they suggest that CEA sensitivity and specificity was 58.3% and 85.7% respectively. There is study achieved in 2003 by (Jolanda et al., 2014) [18] showed CEA has sensitivity of 40%-75% and specificity was 90% in colorectal cancer patients group.

Another study by Ebrahimzadeh et al., (2005) [19] showed the sensitivity and specificity were lower than that existing in this present study. They found that CEA give positive only in serum 37.7% of patients with colorectal cancer. Another study done in china by Chao et al (2001) [20] they found CEA marker give positive only in 29.2% of patients with colorectal cancer. In comparison with previous studies, modern study in Al-Azher university achieved by (Eman et al., 2013) [21] found CEA sensitivity and specificity in colorectal cancer patients group were 65.71% and 88.89% respectively.

There are different values of sensitive and specific for CEA in colorectal cancer patients group between these studies and the present study. By comparing the values in 1996 as 43% the sensitivity and 87% specificity, in 2002 in which the sensitivity was 44% and the specificity 87%, in 2003 the sensitivity was 40-75% and specificity was 90%, in 2007 were the sensitivity 58.3% and 85.7% and in 2013 found the sensitivity 65.71 and the specificity 88.89. the reason for this different as believe the technology in advance from year to year and the technique of kit procedure also in advance therefore the sensitivity and specificity of CEA will increase year after year.

Although CEA has low value in diagnosis of the colorectal cancer in primary stages, it is widely used in diagnosing this type of cancer (Nilsson et al., 1992)[22]. CEA give seropositive in about 90% patients with colorectal cancer and their elevation considered one of most important signals to diagnose the colorectal malignant tumors (He Z et al., 2010) [23].

Also CEA give seropositive in certain healthy individuals but their level is 60-fold lower than malignant tumors (Michael, 2001)[24]. Study done by (Samir, et al., 2001) [25] explained the reason of overexpression of CEA, he suggested that this overexpression occur to protect the malignant cells in tumor from programmed cells death(apoptosis).

CEA is good marker for colorectal cancer and other types of cancer, it has good stability, it is very restricted in its expression in non-malignant tissues and expression in high levels in malignant tissues. In healthy individuals ,large ratio of CEA produced in apical surface of columnar cells and exfoliated into gut lumen and disappear with feces, therefore small amount of CEA normally exist in blood of healthy individuals. In malignant cells of colorectal cancer, these cells lost their polarity and continuously exfoliate CEA as one component of plasma membrane in high levels. This lead to form vesicles derived from cell membrane, then these vesicles drive by lymph and blood vessels to reach the blood (Low and Young, 1988)[26].

Sensitivity and specificity of serum CEA in adenomatous polyp patients group.

The sensitivity. and specificity of CEA in adenomatous polyp patients group is 67.36% and 77.52% respectively as shown in figure(4-4). According to figures (4-3) and (4-4), the sensitivity and specificity of CEA in colorectal cancer patients group were higher than that found in adenomatous polyp patients group.

Study conducted by (Marshall et al., 2009)[27] measured the sensitivity and specificity of CEA in adenomatous polyp patients group and they found it 11% and 95% respectively, as compared with study done by Johanna Louhimo et al., (2002) [28] who found the sensitivity and specificity of CEA in colorectal cancer patients group are 43% and 87% respectively.

Serum CEA concentration in colorectal carcinoma patients group and adenomatous polyp group.

CEA concentration in colorectal carcinoma. patients group and CEA concentration in adenomatous polyp patients group were shown in figures (4-1) and(4-2) respectively.

According to the figure (4-1), there is increase in concentration of CEA in colorectal carcinoma patient group when compared with colitis group. This study agrees with report of American Association for Cancer Research (2010)[29] which confirms the high concentration of CEA in patients with colorectal cancer. Friederichs J et al., (2007) [30] also found the concentration of CEA was elevated in patients with colorectal carcinoma. There is report by Wang JY et al., (2007) [31] found that CEA concentration increase in patients with colorectal carcinoma in comparison with control group. There are no studies that disagree with our result of increase CEA concentration in colorectal carcinoma group.

According to the figure (4-2), there is significant increase in concentration of CEA in adenomatous polyp patients group in comparison with colitis group. This study agrees with results done by Crichlow et al., (1970) [32] explained that CEA concentration was elevated in patients with colorectal adenomatous polyps. Also agrees with study done by Mackie et al., (1962)[33]who explained that CEA concentration increase in patients with adenomatous polyp in comparison with colitis group.

From figures(4-1) and(4-2), it is observed that the CEA concentration in carcinoma patients group is higher than CEA concentration in adenomatous polyp patient group. The concentration of CEA in colorectal cancer patients group and adenomatous polyps patient group increase significantly as compared with colitis group. The highest level was in colorectal cancer, i.e. there are correlation between degree of dysplasia and level of CEA; therefore, measure of CEA was most common to diagnose colorectal cancer (Marshall et al., 2009) [27] . Also it agrees with study done by Daniel FH et al(2010) [34] found that CEA elevated in serum of patients with colorectal cancer and they found its most benefit to diagnose the colorectal cancer. This result agrees with study done by Jing Mead et al., (2011)[35] which found the CEA concentration was high in colorectal adenocarcinoma as compared with adenomatous polyp patient group. Also this result agrees with our study which found the CEA concentration in patients with adenomatous polyps were lower than that found in colorectal carcinoma patients group.

The mechanism that lead to appearance of the CEA concentration in the blood is the same in both the colorectal cancer and adenomatous polyps, but there is difference in level of expression and rate of exfoliation. At first, through transformation of non-malignant cells to malignant cells in adenomatous polyp some of markers will appear in blood such as CEA which starts expression as compound of plasma membrane, one suggestion to explain this expression is to enable malignant cells to protect themselves from programmed cell death(apoptosis)(Samir et al., 2001) [25].

Concentration of serum TAG 72 in colorectal, adenomatous polyp and colitis patients group.

TAG concentration increase significantly in colorectal carcinoma patients group in comparison with colitis group as shown in figure(4-3). This study agrees with a study by Goral V et al., (2007) [36] which found that the level of TAG increase in patients with colorectal cancer. Also This study agrees with a study by Nakayama T et al,.(1997)[37] which found the concentration of TAG increase in patients with colorectal carcinoma.

TAG concentration statically increase in adenomatous polyp patients group when compared with colitis group as shown in figure(4-4). This study agrees with Barbara et al(2015)[38] who found. TAG 72 concentration begin to elevate in pre-malignant colonic conditions such as in colorectal adenomatous polyps cases.

From the two figures(4-3)and (4-4) it is concluded that TAG concentration in colorectal patients group is higher than adenomatous polyp patients group. According to our knowledge there was no previous studies to include this result, but there are studies such as which published in report of AACR (2010)[40] and study by Umar SB et al(2010) [39] which studied the relationship the TAG concentration in familial adenomatous polyposis.

From the two figures(4-1) and (4-3) it is concluded that the concentration of both CEA and TAG significantly increase in colorectal cancer patients group when compared with colitis group . This result agrees with a study byLindmark G et al., (1996) [40] which(found that in colorectal cancer both CEA and TAG increase significantly. Also [41] Xavier et al., (1992) were found a correlation between colorectal cancer development and elevated CEA and TAG 72. Recent study by Huang et al., (2014)[42]also agrees with our results.

From the two figures(4-2) and (4-4) it is concluded that the concentration of TAG and CEA increase significantly in adenomatous polyp patients group when compared with colitis group.

Sensitivity and specificity of serum TAG 72 in colorectal. carcinoma patients group and colorectal adenomatous polyp patients group.

The sensitivity and specificity of TAG in colorectal cancer patients group. The sensitivity and specificity are 88.52% and 100% respectively as shown in figure(4-5). A study by Eman et al (2013)[21]found the sensitivity and specificity of TAG in colorectal cancer patients group were 82.86% and 100% respectively.

The sensitivity and specificity of TAG in colorectal adenomatous polyp patients group were 80.32% and 98.36% respectively as shown in figure(4-6) .

Two figures (4-5) and (4-6) showed that the sensitivity and specificity of TAG 72 in colorectal cancer patients group were higher than these found in adenomatous polyp patients group.

Complementary between both markers in serum.

Not all patients with colorectal carcinoma can be confirmed with CEA marker; therefore, TAG 72 is good choice to confirm diagnosis of colorectal carcinoma. There are reports which recommend to measure CEA and TAG 72 level in serum and this can increase the accuracy because 30% cases were positive with TAG 72 and negative for CEA while 30% cases were negative for TAG 72 and positive for CEA (Fiorella et al., 1991)[43].

Discussion of tissues

Sensitivity and specificity of TAG 72 in tissues sections of colorectal cancer patients group and colorectal adenomatous patients group.

The sensitivity and specificity of TAG 72 in colorectal cancer patients group were 80.00% and 94.29% respectively as shown in table(4-7).

A study indicated that the sensitivity of TAG 72 in colorectal cancer patients group were 28%-67%(Mattar et al., 2001)[44]. Other study by (Zheng et al., 2001)[45]found the sensitivity of TAG 72 was 32.27%. A study achieved by Xavier et al (1994)[41]showed TAG 72 sensitivity in colorectal carcinoma patients group was 61%.

According to table(4-7), the sensitivity and specificity of CEA were 57.15% and 80.00% respectively. There is study was achieved by (Xavier et al., 1994)[41] found the sensitivity of CEA was 31%. Other study achieved by (Zheng et al., 2001)[45]found the sensitivity of CEA was 29.2%, while the sensitivity and specificity were 34.29% and 85.71% respectively.

According to table(4-7) the sensitivity of TAG 72 was higher than sensitivity of CEA and this result agrees with a study by (Ychou et al., 1992)[46].

According to table(4-7), in TAG 72 the sensitivity and specificity were in colorectal cancer patients group more than which found in colorectal adenomatous polyp patients group. This result agrees with study by (Mattar et al., 2002) [44]which found the sensitivity of TAG 72 in colorectal cancer patients group higher than in colorectal adenomatous polyp patients group.

According to table(4-7), CEA the sensitivity and specificity were in colorectal cancer patients group more than those found in colorectal adenomatous polyp patients group.

According to table(4-7), the TAG 72 had sensitivity and specificity higher than CEA in colorectal cancer patients group. Also in colorectal adenomatous patients group the value of sensitivity and specificity were in TAG 72 more than CEA.

Comparative study of colorectal cancer and colorectal adenomatous polyp patients group with colitis patients group in negative and positive cases.

Table(2-8) showed that the total number of colorectal cancer cases were 35, TAG 72 were positive in 28 cases (80%) and 7 cases (20%) give negative for TAG 72. This result agrees with study by (Guadagnie et al., 1991)[47]by used IHC studies found that more than 80% of patients with colorectal cancer give positive to TAG 72 and another study found that TAG 72 overexpressed in high levels in adenocarcinoma and rarely in normal tissues (Zheng et al., 2001)[45]. The cases number which give positive for CEA were 20 (57%) and the negative cases were 15 cases (43%). This ratio is lower than ratio of study done byEbrahim et al., (2016)[48]found that CEA overexpression occur in about 90% of patients with colorectal cancer .According to this result, it can be concludes that statically increased significance of TAG 72 as compared with CEA marker (x^2=24.242 and $p<0.05$).

Table(2-8) showed that the total number of colorectal adenomatous polyp cases included in this study were 35 cases. The cases which give positive for TAG 72 were 19 case (54%), the existing TAG 72 in colorectal adenomatous polyp cases suggested it as early marker to diagnose the colorectal cancer (Fiorella et al., 1994)[49]. The present study agrees with study by (Barbara et al., 2015)[38] which suggest that 5%-75% of patients with colorectal adenomatous polyp give positive for TAG 72. The cases which give positive for CEA were 12 cases (34%). This present result showed the number of cases which give positive for TAG 72 more than CEA in colorectal adenomatous polyp cases(x^2=2.837 and $p<0.05$). according our knowledge there is no previous studies included this result.

Also table(2-8) showed the total number of colitis cases used in this study were 35 cases. Only 2 cases (6%) from total cases give positive for TAG 72 and this result agrees with a study by (Wanebo et al., 1978) [50] found that TAG 72 may be positive in about 3% of patients with benign disease. five cases (14%) give positive to CEA. This result agrees with study achieved by (Fiorella., 1991)[43]who found 10%-15% of patients with benign disease give positive for CEA. In comparison with the results of both CEA and TAG 72 showed there is no significance different between both markers in colitis group (x^2=1.429and $p<0.05$).

The grades of colorectal carcinoma cases for positive and negative CEA and TAG 72 markers.

Eleven out of 16 well differentiated (grade I) colorectal carcinoma cases were positive for TAG 72 (68.75%),while 14 cases out of 16 moderately differentiated carcinoma cases(grade II) were positive for TAG 72 (87.5%) and 3 out of 3 poorly differentiated cases were positive for TAG 72(100%) as shown in table(4-9). This result agrees with study achieved by Zheng et al., (2001) [45] which found TAG 72 give positive in poorly differentiated cases as percentage more than well and moderately differentiated carcinoma.

Eight out of 13 well differentiated colorectal carcinoma (grade I) cases were positive for CEA (61.53%),while 10 cases out of 19 moderately differentiated carcinoma cases(grade II) were positive for CEA (52.63%) and 2 out of 3 poorly differentiated cases were positive for CEA (66.66%) as shown in table(4-9). This result agrees with study achieved by (Zedan et al., 2001)[45] which found that CEA more abundant in poorly and moderately than well differentiated tumor cases.

By comparing the percentages of cases which give positive for both markers with grades shown the percentage increase from well to poorly differentiated as shown in table(4-9). This result disagrees with study by Mario et al., (1996) [63] on serum level which found CEA level influence by grade while TAG 72 don't influence.

Complement between both markers in tissue.

Number of cases included in the present study give positive in TAG 72 while negative for CEA. Also number of cases give negative for TAG 72 while positive for CEA. This result agrees with study by Magdalena et al., (2014)[51]which recommended using TAG 72 and other marker such as CEA to diagnosis colorectal carcinoma. Also a study by Eman et al., (2013) [21]found using TAG 72 and CEA increases accuracy of colorectal carcinoma diagnosis.

CONCLUSION

Serum TAG 72 is more sensitive and specific than CEA in detecting patients with colorectal carcinoma, Tissue TAG 72 is more sensitive and specific than CEA in detecting patients with colorectal carcinoma, Biomarkers may aid in differentiating benign from malignant carcinoma, Measurement of the level of biomarkers in the serum is better than the tissue in the detection and differentiation between types of tumors, Serum biomarkers are reliable markers in detecting colorectal carcinoma and Complementary use of CEA and TAG 72 give more accurate results.

REFERENCES

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, (2017). CA Cancer J Clin ;67:7-30.

2 Leggett, B. and Whitehall, V.. Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology 2010; 138(6): 2088-2100.

3 Coppedè, F., Lopomo, A., Spisni, R. and Migliore, L. . Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. World journal of gastroenterology: WJG 2014; 20(4): 943.

4 Duffy, MJ. ; van Dalen, A. ; Haglund, C., et al. Clinical utility of biochemical markers in colorectal cancer: European Group on tumour Markers (EGTM) guidelines. Eur J Cancer2003; 39: 718-737.

5 Hashiguchi, MD, M. Kasai, MD, T. Fukuda, MD, T. Ichimura, MD, T. Yasui, MD, and T. Sumi, MD (2016)Serum carcinoembryonic antigen as a tumour marker in patients with endometrial cancer Curr Oncol.; 23(5): e439–e442.

6. Wiratkapun, S. ; Kraemer, M. ; Seow-Choen, F. ; Ho Yh, Eu Kw..High preoperative serum carcinoembryonic antigen predicts metastatic recurrence in potentially curative colonic cancer: results of a five-year study. Dis Colon Rectum2001;44:231-5.

7. Carpelan-Holmstrom, M., Louhimo, J.; Stenman, UH.; Alfthan, H. Jarvinen, H.; Haglund, C .CEA, CA 242, CA 19-9, CA 72-4 and hCGbeta in the diagnosis of recurrent colorectal cancer. Tumour Biol 2004; 25(5-6):228-348.

8. Ayude D, Francisco Javier Rodríguez-Berrocal1, José Ayude, Sonia Blanco-Prieto, Lorena Vázquez-Iglesias Marta Vázquez-Cedeira and María Páez de la Cadena1. Preoperative serum CA 72.4 as prognostic factor of recurrence and death, especially at TNM stage II, for colorectal cancer: BMC Cancer 2013; 13:543.

9. Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. Global cancer statistics. CA: a cancer journal for clinicians 2011; 61(2): 69-90.

10. Roy, H. K. and Bianchi, L. K. Differences in colon adenomas and carcinomas among women and men: potential clinical implications. Jama 2009; 302(15): 1696-1697.

11. Zisman, A. L., Nickolov, A., Brand, R. E., Gorchow, A. and Roy, H. K. (2006). Associations between the age at diagnosis and location of colorectal cancer and the use of alcohol and tobacco: implications for screening. Archives of internal medicine 166(6): 629-634.

12. J. Ferlay a,⇑, E. Steliarova-Foucher a, J. Lortet-Tieulent a, S. Rosso b, J.W.W. Coebergh c,d, H. Comber e, D. Forman a, F. Bray a. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012; European Journal of Cancer (2013) 49, 1374–1403.

13. Ricchi, P. ; et al. (1999).Minireview: Nonsteroidal anti-inflammatory drugs in colorectal cancer: from prevention to therapy. Br J Cancer, 88(6): p. 803-7.

14. Edwards, BK.; Howe, HL. ; Ries, LAG.et al. (2002).Annual report to the nation on the status of cancer, 1973–1999, featuring implications of age and aging on U.S. cancer burden. Cancer ; 94 2766–92.

15. Johanna Louhimo ; Monika Carpelan-Holmström ; Henrik Alfthan ;Ulf-Håkan Stenman ; Heikki ,J. Järvinen ; Caj Haglund. Serum HCGβ, CA 72-4 and CEA are independent prognostic factors in colorectal cancer 2002; Volume 101, Issue 6, pages 545–548, 20.

16. Carpelan-Holmström ,M. ; Haglund ,C.; Roberts, PJ. Differences in serum tumour markers between colon and rectal cancer. Dis Colon Rectum, 1996; 39: 799-805.117.

17. Hashem, A. ; Dbouk ; Ayman Tawil ; Fahd Nasr ; Loucine Kandakarjian and Raghida Abou-Merhi..Significance of CEA and VEGF as Diagnostic Markers of Colorectal Cancer in Lebanese Patients.The Open Clinical Cancer Journa l2007; Vol 1 ; 1:5.

18. Jolanda Stiksma, Diana C. Grootendorst, Peter Willem G. van der Linden. CA 19-9 As a Marker in Addition to CEA to Monitor Colorectal Cancer 1Clinical Colorectal Cancer Elsevier Inc., 2014; Vol. 13, No. 4, 239-44.

19. Ebrahimzadeh, ME. ; Miri, MR. ;Fattahi, E.(2005).The prognostic value of preoperative serum levels of CEA and CA 242 in patients with colorectal cancer.Vol. 4 No .

20. Chao X, Wen H, Ji Zong Z, Dong Z, Dong P, Yu L and Zhang Q: The prognostic value of preoperative serum levels of CEA, CA19-9 and CA72-4 in patients with colorectal cancer. World J Gastroenterol. 2001;,7(3):431-434.

21. Eman M. I. Youssef1, Gehan H. Ewieda1, Haneya A. A. Ali, Amany M. Tawfik2, Wafaa Mohi El-deen Abd El-fatah1, Amgad A. Ezzat, Rehab M. Elsaid Tash5, Nashwa El-Khouly(2013). Comparison between CEA, CA 19-9 and CA 72-4 in Patients with Colon Cancer; International Journal of Tumor Therapy 2013, 2(1): 26-34.

22. Nilsson, O. ; Johansson, C. Glimelius, B.; Norgaard Pedersen B. Andren Sandberg, A. ; and Lindholm, L .Sensitivity and specificity CA242 in gastro-intestinal cancer.A Comparison with CEA,CA50 and CA19-9. Br J Cancer 1992; 65,215-221.

23. He Z, Shi C, Wen H, Li F, Wang B, Wang J. The potential ofcarcinoembryonic antigen, p53, Ki-67 and glutathione S-transferase-π clinico-histopathological markers for colorectal cancer. Journal ofBiomedical Research 2010; 24:51-57.

24. Michael J. Duffy(). Carcinoembryonic Antigen as a Marker for Colorectal Cancer: Is It Clinically Useful?; Clinical Chemistry 2001; 47:4 624–630.

25. Samir Zedan, Mosad Morshed1,Wael Khafagy1, Sabry Ahmed1, Fatima El-Hossini2, Mohamed El-Shobaky. Study of Carcinoembryonic Antigen Tissue Expression in Colorectal Cancer; Coloproctology 2001;23:88–93.

26 Low and Young S. Kim(). Release of Carcinoembryonic Antigen from Human Colon Cancer Cells by Phosphatidylinositol-specific Phospholipase C Todd L. Sack, James R. Gum, Martin; The Journal of Clinical Investigation, Inc. 1988; Volume 82, 586-593.

27. Marshall E. Goldberg, Lisa M. Simunovic, Sandra L. Drake, Willys F. Mueller, Jr., and Harland L. VERRILL. Hybridoma, 2009;8(5): 569-575.

28. Johanna Louhimo ; Monika Carpelan-Holmström ; Henrik Alfthan ;Ulf-Håkan Stenman ; Heikki ,J. Järvinen ; Caj Haglund. Serum HCGβ, CA 72-4 and CEA are independent prognostic factors in colorectal cancer 2002; Volume 101, Issue 6, pages 545–548, 20.

29. American Association for Cancer Research (2010).

30 Friederichs J, Gertler R, Rosenberg R, Dahm M, Nekarda H, Holzmann B, et al. Correlation of CK-20-positive cells in peripheral venous blood with serum CEA levels in patients with colorectal carcinoma. World J Surg. 2007;31:2329-34.

31. Wang JY, Lu CY, Chu KS, Ma CJ, Wu DC, Tsai HL, et al(2007). Prognostic significance of pre- and postoperative serum carcinoembryonic antigen levels in patients with colorectal cancer. Eur Surg Res. 2007;39:245-50. Epub; Apr 23.

32. Crichlow, R. W., and White, R. Search for carcinocmbryonic antigen (CEA) in adenomas of the colon. Proc. Am. Assoc. Cancer Res1970; 11:18.

33. Mackie, J. A., Jr., Miller, L. D., and Fitts, W. T., Jr. Polyps and polypoid lesions of the large bowel; surgical considerations. Surg. Clin. North Am. 1962; 42:1451- 1468.

34. Daniel FH, Robert CB, Christopher ED, Herbert FJr, Nancy EK, Jessup MJ, Gershon YL, John SM, Robert GM, Larry N, Peter R, Sheila T, Rodger JW. Tumor Marker Utility Grading System: a Framework to Evaluate Clinical Utility of Tumor Markers. J Natl Cancer Inst 2010; 88: 1456-1466. Biomedica Vol.26, JanBio-11.Doc P. 16 – 19.

35. Mead R, M Duku, P Bhandari and IA Cree. British Journal of Cancer (2011) www.bjcancer.com 105, 239–245.

36. Goral V, Yesilbagdan H, Kaplan A and Sit D . Evaluation of CA 72-4 as a new tumor marker in patients with gastric cancer. Epatogastroenterology; 2007; 54: 1272-5.

37 Nakayama T, Watanabe M,Teramoto T, Kitajima M .CA19-9 as a predictor of recurrence in patients with colorectal cancer. J Surg Oncol 1997;66: 238–43

38 Barbara C. Wolf John C. D'Emilia, Ronald R. Salem, Deborah DeCoste, Henry F. Sears, Leonard S. Gottlieb, Glenn D. Steele, Jr. Detection of the Tumor- Associated Glycoprotein Antigen (TAG-72) in Premalignant Lesions of the Colon Natl Cancer Inst 2015; 81 (24): 1913-1917.

39 Umar SB, DiBaise JK. Protein-losing enteropathy: case illustrations and clinical review. Am J Gastroenterol. 2010;105:43–9.40-Lindmark G, Kressner U,Bergstrom R, Glimelius B.Limited. clinical significance of the serum tumour marker CA 72-4 in colorectal cancer.Anticancer Res 1996;16:895–8.

40 American Association for Cancer Research(2010).

41 Xavier Filella, Jose Fuster, Rafael Molina, Juan Jose Grau, Luis Grande & Antonio M. Ballesta. Tag-72, CA 19.9 and CEA as Tumor Markers in Gastric Cancer; Acta Oncofogica 1994;VoI. 33, No. 7, pp. 747-751.

42 Huang ZB, Zhou X, Xu J, Du YP, Zhu W, Wang J. Prognostic value of preoperative serum tumor markers in gastric cancer. World J Clin Oncol 2014;5:170–6.

43 Fiorella Guadagni, Mario Roselli, Maurizio Cosirnelli, Ernest Mannella, Manfred Tedesco, Francesco Cavaliere, Antonio Grassi, Maria Rosaria Abbolito, Ph.D, John W. Greiner, PhD, and Jeffrey Schlo. TAG-72 (CA 72-4 Assay) as a Complementary Serum Tumor Antigen to Carcinoembryonic Antigen in Monitoring Patients with Colorectal Cancer1991; Grant A.I.R.C.

44 Mattar R, Alves de Andrade CR, DiFavero GM, Gama-Rodrigues JJ, Laudanna AA. Preoperative serum levels of CA 72-4, CEA, CA 19-9, and alphafetoprotein in patients with gastric cancer. Rev Hosp Clin Fac Med Sao Paulo2002; 57: 89-92.

45 Zheng CX, Zhan WH, Zhao JZ, Zheng D, Wang DP, He YL, Zheng ZQ. The prognostic value of preoperative serum levels of CEA, CA19-9 and CA72-4 in patients with colorectal cancer. World J Gastroenterol 2001,;7(3):431-434.

46 Ychou M, Tuszinski T, Pignon JP, et aI. Adenocarcinomesgastriques: comparaison du CA 19-9 et de I'antigine carcinoembryonnaire pour le diagnostic des recidives apres traitement chirurgical. Gastroenterol Clin Biol; 1992; 16: 848-52.

47 Guadagni F, MD, Mario Roselli, , Teresa Aamto, Maurizio Cosimelli, Erenest Mannella, MD, Manfred Tedesco, Antonio Grassi, Vincenzo Casale, MD, Franceso Cavaliere, Johnw. Greiner , and Jeffrey Schlom(1991), Clinical Evaluation of Serum Tumor-Associated Glycoprotein-72 as a Novel Tumor Marker for Colorectal Cancer Patients; Journal of Surgical Oncology Supplement 2: 16-20.

48 Ebrahim Eftekhar,Hajar Jaberie, Fakhraddin Naghibalhossaini(2016).

49 Fiorella Guadagni, Mario Roselli, Maurizio Cosimelli, Antonella Spila, Francesco Cavaliere, Raffaella Arcuri, Maria Rosaria Abbolito, John W. Greiner, Jeffrey Schlom,. .Biologic Evaluation of Tumor- Associated Glycoprotein-72 and Carcinoembryonic Antigen Expression in Colorectal Cancer, Part I 1994; volume. 37 no.2 .

50 Wanebo HJ, Rao B, Pinsky CM, Hoffman RG, Stearns M, Schwartz MK, Oettger HF. Preoperative carcinoembryonic antigen levels as a prognostic indicator of colorectal cancer. N Engl J 1978; Med 299:448-45.

51 Magdalena Świderska, Barbara Choromańska, Ewelina Dąbrowska, Emilia Konarzewska-Duchnowska, Katarzyna Choromańska, Grzegorz Szczurko, Piotr Myśliwiec, Jacek Dadan, Jerzy Robert Ładny, Krzysztof Zwierz. The diagnostics of colorectal cancer ;Contemp Oncol (Pozn) 2014;18 (1): 1–6