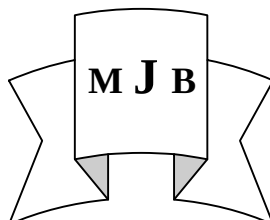


Immunohistochemical Study of CEA and BCL2 Expression in Colorectal Adenocarcinoma and its Correlation with Some Pathological Parameters

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Abstract

Background :- Colorectal cancer is the second most prevalent cancer and the fourth leading cause of death[1]. In Iraq and according to the Iraqi cancer registry reports in 2002 , the incidence of colorectal carcinoma was 4.55% of whole body malignancy, & it is the seventh cause of death from cancer[3]. Immunohistochemical studies have revealed that CEA is a product of normal as well as cancerous colonic cell and that the difference in CEA content between the cancerous and the normal colorectal mucosa is quantitative and not qualitative[9-11], Expression of bcl-2 has been studied in a number of human tumors, including colorectal carcinoma. Non-small-cell lung carcinomas[18] breast carcinomas[19,20] and neuroblastomas[21] all express bcl-2, and studies have been published correlating expression with survival. Similarly, a number of studies[22-26] have been published evaluating the clinical use of bcl-2 immunohistochemical expression as a prognostic factor.

Aim of the study :- The aim of our study was to estimate possible correlations between the CEA and BCL-2 immunohistochemical expression and some pathological parameters in colorectal adenocarcinoma like grade , stages and site of tumor

Material and method:- thirty nine patients of colorectal carcinoma studied in Al- Diwanya teaching hospital during a three years period (July 2008 - December 2010) were studied. There were 30 males and 9 females with a median age of 58 years (range 26-90 years). A manual avidine-biotin-peroxidase complex procedure was used in the immunohistochemical analysis (DakoCytomation, Copenhagen, Denmark).

Result :- all the studied cases were positive for CEA (100%) and out of 39 cases there were only 23(58.9%) cases positive for BCL2 expression , also this study showed significant relationship between expression of CEA and grades and site of tumor while BCL2 significantly correlated with grades and stages of the tumor.

Conclusion :- From the present study we may conclude that CEA and BCL-2 have a similar pattern of expression in primary colorectal adenocarcinoma although BCL-2 expression in colorectal adenocarcinoma is more restricted than CEA expression

الخلاصة

الهدف من البحث هو دراسة التعبير المناعي النسيجي الكيميائي لجيني أل BCL-2 و CEA كمؤشر للتغيرات الطارئة في الخلايا السرطانية لأورام القولون والمستقيم و معرفة ترابط هذا التغير مع درجة تمايز الورم ودرجة انتشاره و موقع الورم . تمت دراسة 39 عينة من سرطان القولون في مختبرات مستشفى الديوانية التعليمي لمرضى أجرو استئصال القولون للفترة من تموز ٢٠٠٨ إلى كانون الثاني ٢٠١٠ . أظهرت الدراسة إن التعبير النسيجي لجين أل -CEA (١٠٠%) كان أكثر من التعبير النسيجي لجين أل -BCL2 (٥٨,٩%) مع وجود ترابط في التعبير النسيجي لجين أل -CEA مع مجموعات تمايز المرض وموقع انتشاره بينما كان التعبير النسيجي لجين أل -BCL2 مترابطاً مع درجتي تمايز المرض وانتشاره ، مما يدل على أن لهذين الجينين دوراً أساسياً في تقييم سرطان القولون والمستقيم المستقبلية وانتشاره وتحديد أهمية استجابته للعلاج.

Introduction

Colorectal cancer is the second most prevalent cancer and the fourth leading cause of death[1].

In Iraq and according to the Iraqi cancer registry reports, the colonic cancer represented about 4.7% of all malignant primary tumors registered during the period from 1995-1997, while rectal malignancy represented about 3.4% [2]. In 2002, the incidence of colorectal carcinoma was 4.55% of whole body malignancy, & it is the seventh cause of death from cancer[3]. Also the distribution of colorectal cancer according to the race in Iraq was 86.6% Arabs &13.3% Kurds with a ratio of Arabs: Kurds 6.6:1[4-5].

CEA is an in completely defined glycoprotein 180000 Daltons[6], it was first isolated from human fetal intestine and adult colon cancer tissue by Gold and Freedman in 1965 [7,8]. It was originally found in colorectal carcinomas[6], Immunohistochemical studies have revealed that CEA is a product of normal as well as cancerous colonic cell and that the difference in CEA content between the cancerous and the normal colorectal mucosa is quantitative and not qualitative[9-11]. More recently, abnormality in surface expression of CEA was shown to be a characteristic of the colonic and gastric neoplastic cells[12].

Bcl-2 (B-cell lymphoma/leukemia-2) was initially identified at a breakpoint in a chromosomal translocation (t14:18) that occurs in human B-cell lymphomas. Bcl-2 is a known inhibitor of apoptosis[13,14], and has also been shown to block chemotherapy-induced apoptosis[15]. The ability of bcl-2 to inhibit apoptosis depends on the intracellular balance among a number of its family members, including BAG1, Bad, Bax, bcl-xL and bcl-xS. Bcl-xL, like bcl-2, functions as an inhibitor of apoptosis, whereas bcl-xS

serves as a dominant negative inhibitor of bcl-xL and bcl-2 [16]. The bcl-2 binding protein (BAG1) inhibits apoptosis whereas the Bad and Bax proteins promote apoptosis[17]. Expression of bcl-2 has been studied in a number of human tumors, including colorectal carcinoma. Non-small-cell lung carcinomas[18] breast carcinomas [19,20] and neuroblastomas[21] all express bcl-2, and studies have been published correlating expression with survival. Similarly, a number of studies [22-26] have been published evaluating the clinical use of bcl-2 immunohistochemical expression as a prognostic factor.

Material and Method

Thirty nine consecutive patients undergoing potentially curative resection of a colorectal adenocarcinoma in a single unit who had referred to Al-Diwaniya Hospital during a three years period (July 2008 - December 2010) were studied. There were 30 males and 9 females with a median age of 58 years (range 26-90 years).

On histopathological and surgical assessment and according to Modified Dukes staging system [27], five (12.8%) of the tumors were classified as Dukes stage A, 19 (48.7%) as Dukes stage B and 15 (38.5%) as Dukes stage C. Twenty (51.2%) cases were graded as well differentiated, 17(43.5%) moderately differentiated and two (5.1%) poorly differentiated. Ten (25.6%) of the primary tumors were in the right colon (caecum, ascending and right transverse), 29 (74.3%) in the left colon (left transverse, descending, sigmoid and rectum).

A manual avidine-biotin-peroxidase complex procedure was used in the immunohistochemical analysis (Dako Cytomation, Copenhagen, Denmark). Positive control sections were colonic cancers known to express both

antigens. Negative control sections consisted of either no first antibody (anti-CEA or anti-BCL2) or neither first nor second antibodies being applied.

The BCL2 and CEA expression was evaluated relative to the percentage of the tumor cells with cytoplasmic reaction (usually brown) , based on the proportion of the total number of tumor cells: as follow < 25% = score 1 , 25- 49% = score 2, 50 - 75%= score 3 and > 75% = score 4⁽²⁸⁾. Data were analyzed using the SPSS verge 17 software and the chi-square was used where P value ≤ 0.05 .

Result

The staining pattern was similar for both the anti- CEA and anti-BCL2 antibodies, being most pronounced on the cytoplasmic , but few cases express CEA at the luminal cell membrane in areas of tumor with gland formation [figure 1]. All 39 colorectal cancer sections stained positively for CEA whereas 23/39 sections stained positively for BCL2 (58.9%).

The score 3 and 4 of immunohistochemical staining of the tumors was higher with anti-CEA than anti-BCL2 as (10.3%) of anti-CEA and (5.1%) of anti-BCL2 in score 3 while (56.4%) of anti-CEA and (30.7%) of anti-BCL2 in score 4 (table 1).

Score 3 and 4 CEA expression were more in well rather than moderately

differentiated cases (16 of 20 versus 9 of 17) (table2). While BCL2 expression were more in poor rather than well differentiated cases (100% versus 25%) and the scores 3 and 4 BCL2 expression were more in poor rather than well differentiated cases (50% versus 35% versus 25%) , with statistically relationship fore the both markers with the grades of the tumor (table 3).

CEA expression immunohistochemically varied with the site of the primary tumor, right sided colonic tumors showing less intensity of CEA expression than the left sided, Six of the 10 right sided lesions were score 3 and 4 whereas 20 cases of the 29 lesions in the left colon were score 3 and 4 with statistically relationship (P = 0.0222) (table 2), for BCL2 expression were 5 of 10 in the right colon and 9 of 29 in the left colon are graded in score 3 and 4 with no significant relationship (P > 0.05) (table 3).

CEA expression immunohistochemically were more intensity in Dukes C than A and B (score 4 is as 80% in C, 60% in A and 36.8% in B) with no statistically significant relationship (P = 0.2119) (table 2), while in BCL2 expression there is a statistically significant relation ship with the stages of the tumor in spite of that its expression is zero in A , 52.6% in B and 13.3% in C (P = 0.0019) (table 3).

Table 1 Expression of CEA and BCL2 in colorectal cases.

Marker	Scores					Total
	0	+1	+2	+3	+4	
CEA	0	6 (15.4%)	7 (17.9%)	4 (10.3%)	27 (56.4%)	39
BCL2	16 (41%)	2 (5.1%)	7 (17.9%)	2 (5.1%)	12 (30.7%)	39
$X^2 = 21.6078$ $P = 0.00009$						

Table 2 Scoring system of expression of CEA in relation to the pathological parameters of colorectal carcinoma

Features	CEA (-Ve)	CEA (+Ve)				X ²	P
	0	+1	+2	+3	+4		
Grades							
GI	0	0	3(15%)	4(20%)	13(65%)	13.2773	0.0387
GII	0	5(29.4%)	3(17.6%)	0	9		
GIII	0	1(50%)	1(50%)	0	0		
Sites							
Left	0	2(6.8%)	7(24.3%)	2(6.8%)	18(62%)	9.5972	0.0222
Right	0	4(40%)	0	2(20%)	4(40%)		
Stages							
Dukes A	0	0	1(20%)	1(20%)	3(60%)	8.3728	0.2119(NS)
Dukes B	0	4(21%)	5(26.3%)	3(15.7%)	7(36.8%)		
Dukes C	0	2(13.3%)	1(6.7%)	0	12(80%)		

Table 3 Scoring system of expression of BCL2 in relation to the pathological parameters of colorectal carcinoma

Features	Bcl-2 (-Ve)	Bcl-2 (+Ve)				X ²	P
	0	+1	+2	+3	+4		
Grades							
GI	15(75%)	0	4(20%)	1(5%)	0	28.299	0.0003
GII	1(5.8%)	2(11.7%)	3(17.6%)	1(5.8%)	10(58.8%)		
GIII	0	0	0	0	2(100%)		
Sites							
Left	12(41.3%)	1(3.4%)	7(24.1%)	1(3.4%)	8(27.5%)	4.0345	0.4013(NS)
Right	4(40%)	1(10%)	0	1(10%)	4(40%)		
Stages							
Dukes A	3(60%)	0	0	2(40%)	0	24.325	0.0019
Dukes B	6(31.5%)	1(5.2%)	2(10.4%)	0	10(52.6%)		
Dukes C	7(46.6%)	1(6.6%)	5(33.3%)	0	2(13.3%)		

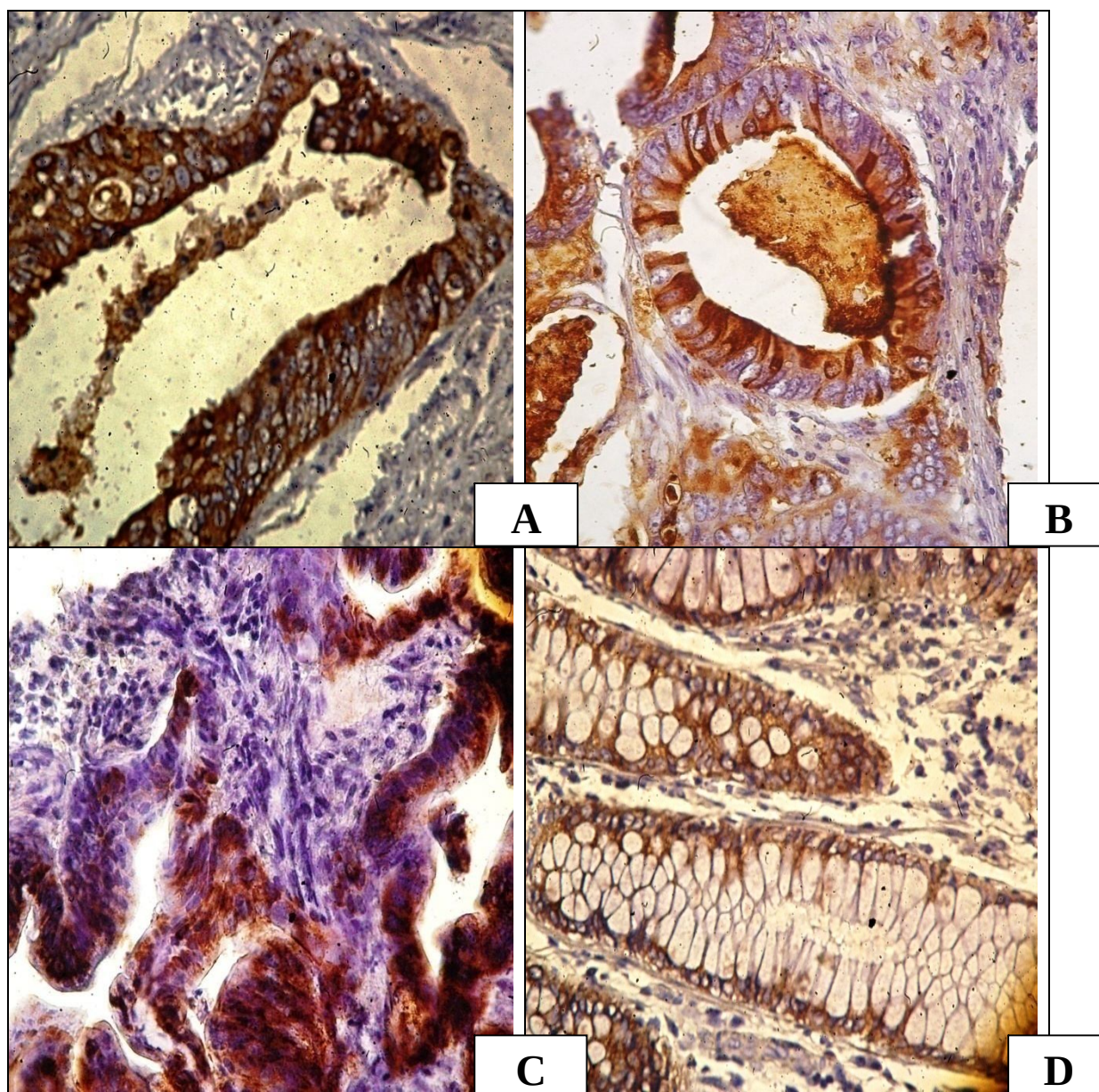


Figure 1 Colonic Adenocarcinoma, 1- CEA Staining in grade I adenocarcinoma , (A) Cytoplasmic , (B) luminal cell membrane 2- BCL-2 Staining (C) Cytoplasmic in grade I adenocarcinoma (D) grade II adenocarcinoma, 40X

Discussion

The identification of molecular markers that provide an insight into the potential behavior or aggressiveness of tumors is a necessary step for the improvement of cancer treatment. Bcl-2 protein product seems to be one of the most promising members of molecular markers to evaluate cancer malignant behavior. The BCL-2 gene is a protooncogene whose protein product inhibits apoptosis. Its role is

associated with keeping cells alive, but not by stimulating them to proliferation, as other protooncogenes do. CEA is considered as one of the most clinically significant tumor marker for colorectal cancer, providing information on prognosis, tumor recurrence and metastasis, In colorectal cancer however, its concentration is increased and the general distribution of the molecule on the cell and cell surface is altered [10-11].

In present study the staining pattern was similar for both the anti-CEA and anti-BCL2 antibodies, being most pronounced on the cytoplasmic but few cases express CEA at the luminal cell membrane in areas of tumor with gland formation in addition to cytoplasmic expression, this in agreement with Davidson B R et al [29] who studied the CEA expression in normal colon, adenomas and adenocarcinoma of the colon and rectum and Contup. C. et al [30] who studied the expression of BCL2 in rectal cancer . They conclude that cytoplasmic staining pattern is more pronounced than the luminal cell membrane in areas of gland formation while Eadie Hydriman et al [31] conclude that CEA cytoplasmic staining expression in poor differentiated cancer and luminal cell membrane is with well and moderately differentiated cancer.

In present study, 58.9% of the colorectal adenocarcinomas were BCL-2 immunohistochemically positive. The prevalence of BCL-2 protein immunocytochemical expression in colorectal cancers varies greatly from one study to another: 16.7% [32], 24% [33], 27% [34], 28.1% [35], 29% [36], 29.5% [30], 43% [37], 45% [38], 46.7% [39], 51.9% [40]. With respect to the location, BCL-2 could not be significantly correlated with the locations, this in agreement with Petrisor .O. et al whose studied the expression of Ki-67 , P53, and BCL-2 in colonic cancer versus rectal cancer and concluded that BCL-2 has no correlation with site of tumor [41]. Also there were a significant correlation between the BCL-2 expression and the grade and stage of the tumor, A significant association between BCL-2 expression and tumor stage and grade was demonstrated in Schwandner. O. et al whose research

was about the P53 and BCL-2 as a significant predictors of survival in rectal cancer [36]. The result pleading, as in the case of the proliferation index, for the support of the general mechanism of colorectal carcinogenesis, without differentiations depending to the intestinal segment involved.

A few reports suggested that the use of CEA as an indicator of second look surgery resulted in complete removal of recurrent colorectal cancer , and some indicated 5-year cures after CEA- direct resection . But strong opposition to the concept of both second look surgery and the use of CEA as an indicator of recurrent cancer has persist, especially in the medical literature [45].

Present immunohistochemistry results of CEA expression in 100% of studied cases further support the findings of previous studies which conclude that CEA is expressed by all primary colorectal cancers[29,31, 42-44]. Also Peter Isacson et al demonstrate 100% expression of CEA in all colorectal carcinoma studied cases by using immunofluorescence technique[25]. Also CEA expression were more in well rather than poorly differentiated cases and in left sided than right sided cases and in metastasized tumor than non metastasized tumor . A correlation between CEA expression in colonic carcinomas and their degree of differentiation has previously been suggested and the association between CEA expression and tumor differentiation is controversial, with some studies showing greatest CEA expression in well differentiated colorectal carcinoma [28,47-48] and others conclude more expression in poorly differentiate colorectal carcinoma [49, 50]. Moreover although Gold P et al conclude that CEA expression of cancers increases from proximal to distal in the

gastrointestinal tract [50] and Yoshinoory Hamada who was studied the immunohistochemical CEA expression in colorectal cancer correlated with CEA level conclude that there is a strong relation ship for the marker tissue expression and elevated serum level with staging of cancer with more expression and high serum level in the metastasis tumor rather than non metastasis one [25].

From the present study we may conclude that CEA and BCL-2 have a similar pattern of expression in primary colorectal cancers and their nodal mtastases although BCL-2 expression is more restricted than CEA. This pattern of antigen expression is of clinical significance. Monoclonal antibodies to CEA or BCL-2 selected for an individual patient or tumour based on immunohistochemical staining of biopsies may allow a greater antibody uptake in tumour tissue and improved tumour targeting for imaging or therapy in patients with colorectal cancer.

Conclusion

From the present study we may conclude that CEA and BCL-2 have a similar pattern of expression in primary colorectal adenocarcinoma although BCL-2 expression in colorectal adenocarcinoma is more restricted than CEA expression. So these genes can be used in determination of the prognosis of colorectal adenocarcinoma and larger epidemiological studies of colorectal carcinoma are warranted for further elucidate the effect of CEA and BCL-2 gene mutation on both the survival rates & the response to treatment in Iraq.

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