Correlation between BCL2 protein expression and clinicopathological parameters of colorectal carcinoma

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الخلاصة:
البحث عن التعبير المناعي النسيجي الكيميائي لجين أل bcl-2 كمؤشر للتغييرات الطارئة في الخلايا السرطانية لأورام القولون والمستقيم. تمت دراسة ٥٠ عينة من سرطان القولون في مختبرات مستشفى الديوانية التعليمي. أظهرت الدراسة وجود ترابط في التعبير النسيجي لجين أل- bcl-2 مع مجموعات تمايز المرض ودرجة انتشاره وكان التعبير أكثر في العينات ذات التمايز القوي (الدرجة الأولى) والمجموعة غير المنتشرة ضمن جدار القولون والمستقيم عنه في العينات ذات التمايز الضعيف (الدرجة الثالثة) والمنتشرة خارج جدار القولون والمستقيم مما يدل على أن لهذا ألجين دورا اساسيآ في تقيم سرطان القولون والمستقيم المستقبلية وانتشاره وتحديد أهمية استجابته للعلاج.

Abstract
This study was conducted to estimate the expression of BCL2 oncoprotein in human colorectal carcinoma and to show its possible correlation to the clinic-pathological parameters. 50 patients of colorectal carcinoma studied in Diwania teaching hospital , out of 50 cases there were only 14 cases positive for bcl2 expression, (10%) of them with strong positive staining pattern, (12%) of them with moderate positive and (6%) with weak positive. Grade I showed (66.7) strong positive staining pattern, while Grade III showed (100%) weak positive staining pattern, also this study showed high expression of BCL2 in stage A,B than stage C,D which were with negative result.

Aim of the study
The aim of our study was to estimate possible correlations between the Bcl-2 expression and some clinicopathological parameters in colorectal carcinoma

Key words:- Colorectal carcinoma, BCL2 oncogene, IHC.

Introduction:-

Cancer is an important problem in public health worldwide, colorectal carcinoma is the third common cancer after the breast cancer in female and bronchus cancer in male and the second leading cause of death among cancer in the western world. The colorectal cancer incidence is 9.4% of all cancer incidence in male and 10.1% in female [(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)].

Pathophysiology colorectal carcinoma is complex and developed in multisteps process in which several gene mutations will occur an coordinate with each other in genotyping and phenotyping outcome. This mutation will occur in many tumor suppressor genes

[APC,DCC,TP53 genes], and proto-oncogenes [K-ras, C-myc gene], leading to the development of sporadic and hereditary colorectal carcinoma [6].

Imunohistochimical study of colorectal cancer has started in 1980 & many type of specific and non specific glycoprotein as a biological marker has been employed on colorectal cancer tissue for the assessment of aggressiveness and prognosis of the disease like CA19-9,EGFR,CDX-2,CEA,Cytokeratine-20, P53[7], Bcl2 and BAX[8].

BCL2: the bcl2-gene encodes for a membrane-associated protein that is present in outer mitochondrial membrane. Additionally it was described in some parts of the endoplasmatic reticulum and nuclear envelope[9,10,11]. Expressed widely during embryonic development, in the adult it is confined to long-lived cells (e.g. stem cell populations, resting B lymphocytes, and peripheral neurons)[12].The biochemical function remains largely unknown, although bcl-2 oncoprotein is known to inhibit programmed cell death[13] ,Furthermore some data support a role in cell growth control via regulation of the Redox System of cells[14,15,16]. Recently a paradoxical inhibition of in vitro cell growth has been reported in several solid tumor cells lines[17]. The bcl-2 gene product was shown to be over-expressed in the 14;18 translocation of human B-cell lymphoma[18], Hodgkin's disease, and reactive lymph nodes[19] and was immunohistochemically demonstrated in breast carcinoma[20,21,22], follicular carcinoma of the thyroid[23], non-small-cell lung cancer[24], hepatocellular carcinoma[25] and neuroblastoma[26]

Material and methods:

This study included fifty cases of colorectal carcinoma studied prospectively, collected randomly in a period from January 2007 to Juan 2010 from AL-Diwannia Teaching Hospital as well as private hospitals. Their samples including excisional biopsies were collected from AL-Diwannia Teaching Laboratories as paraffin embedded blocks. Clinical information were collected from hospital reports including age ,sex , site of tumor , histological type , stage & grade of the colorectal carcinoma. Patient with colorectal carcinoma were grouped according to the site into (26 rectal carcinoma ,24 colonic carcinoma , and according to the sex as 42 male ,8 female , mean age 67.8 ranges from (35-90) years , according to the grades as 7 grade I,17 grade II, 26 grade III, according to the stages as 4 Dukes A, 14 Dukes B, 17 Dukes C ,15 Dukes D **.** Tumor tissues were routinely processed (formalin-fixed and paraffin-embedded) and were classified according to Dukes' classification [28], with an added D-stage for patients with distant metastases, TNM staging system [29] and WHO grading system[30]. A manual avidine-biotin-peroxidase complex procedure was used in the immunohistochemical analysis (Dako Cytomation, Copenhagen, Denmark);the expression is appeared as cytoplsmic staining[31].

Semi quantitative assessment of immunohistochemical staining patterns:-The staining was categorized as follows:

Negative = no immunoreactive cells detectable

Weak positive =fewer than 5% bcl-2 (+)

Moderate positive = 5-50% of tumor cells (+ +)

Strong positive = more than 50% (+ + +)

The semi quantitative evaluation was shown to be highly reproducible since no divergent diagnoses was made in the second assessment[32] . data were analyzed using the SPSS software and the chi-square was used.

Result:-

36 out of 50 cases (72 %) investigated completely lacked immunohistochemically detectable of bcl-2. Weak positive staining pattern of bcl-2-positive cells were found in 3 (6%) of cases(figure 1), moderate staining pattern were found in 6 (12%) of cases (figure2), and strong positive were found in 5 (10%) of cases that investigated (figure3). (table 1).

Table 1: Scoring of bcl2 expression

Score	-Ve	+Ve	+ + Ve	+++Ve	total
NO.	36(72%)	3(6%)	6(12%)	5(10%)	50(100%)

There was no statistically significant relation ship between the bcl2 expression and sex, age, and site of colorectal carcinoma when {p value>0.05} (table 2, 3 and 4) respectively.

In our study noticed 6(85.7%) positive expression in grade I, 6(35.3%) in grade II and 2(7.7%) in grade III. also see 4(66.7%) strong positive in grade I while 2(100%) weak positive in grade III. The strong expression of bcl2 Were seen 4(66.7%)in grade I more than grade II 1(16.7%) while 0(0%) in grade III, all grade III positive cell give weak expression (table 7,8). There was statistically significant relation ship between the grade of tumor and the bcl2 expression and intensity when (p value <0.05). Also in our study seen positive expression 3(75%) in stage A ,10 (71%) in stage B, 1(5.9%) in stage C, all stage D give negative expression , the strong expression of bcl2 were seen in stage A, more than B while negative in C and D (table 5,6), there were statistically significant relation ship between the stage of tumor and bcl2 expression and intensity when (p<0.05).

Table 2:- The correlation between bcl2 expression in colorectal carcinoma and sex group.N=50

Gender	-Ve	+Ve	χ	P
Male	31(73.8%)	11(26,2%)	0.054	NS
Female	5 (62.5%)	3(37.5%)		

Table 3:-The correlation between bcl2 expression in colorectal carcinoma and age groups (N=50)

AGE	-Ve	+Ve	χ	p
≤ 60	21 (72.4 %)	8 (27.6 %)	0.974	NS
>60	15 (71.4 %)	6 (28.6 %)		

Table 4:-The correlation between bcl2 expression in colorectal carcinoma and site of carcinoma. (N=50)

Site	-Ve	+Ve	χ	P
Rectal	16 (61.5 %)	10 (38.5 %)	2.94	NS
Colonic	20 (83.3 %)	4 (16.7 %)		

Table 5:- The correlation between bcl2 expression in colorectal carcinoma and stage of tumors. (N=50)

Stag of tumor	-Ve	+Ve	χ	P
A	1 (25 %)	3 (75 %)	27.41	<0.05
B	4 (28.6 %)	10 (71.4 %)		
C	16 (94.1 %)	1 (5.9 %)		
D	15 (100 %)	0 (0 %)		

Table 6:- the intensity of bcl2 expression in colorectal carcinoma and stages of the tumor.

Stage	+	+ +	+ + +	χ	P
A	0	0	3 (100%)	10.52	< 0.05
B	2 (20%)	6 (60%)	2 (20%)		
C	1(100%)	0	0		
D	0	0	0		

Table 7:-The correlation between bcl2 expression in colorectal carcinoma and grade of tumors (N=50)

Grades	-Ve	+Ve	χ	P
Grade I	1 (14.3 %)	6 (85.7 %)	17.29	<0.05
Grade II	11 (64.7 %)	6 (35.3 %)		
Grade III	24 (92.3 %)	2 (7.7 %)		

Table 8:- the intensity of bcl2 expression in colorectal carcinoma and grade of tumor

Grades	+	++	+++	χ	P
I	0	2 (33.3 %)	4(66.7 %)	11.96	< 0.05
II	1(16.7 %)	4 (66.6 %)	1 (16.7 %)		
III	2 (100 %)	0 (0 %)	0 (0 %)		

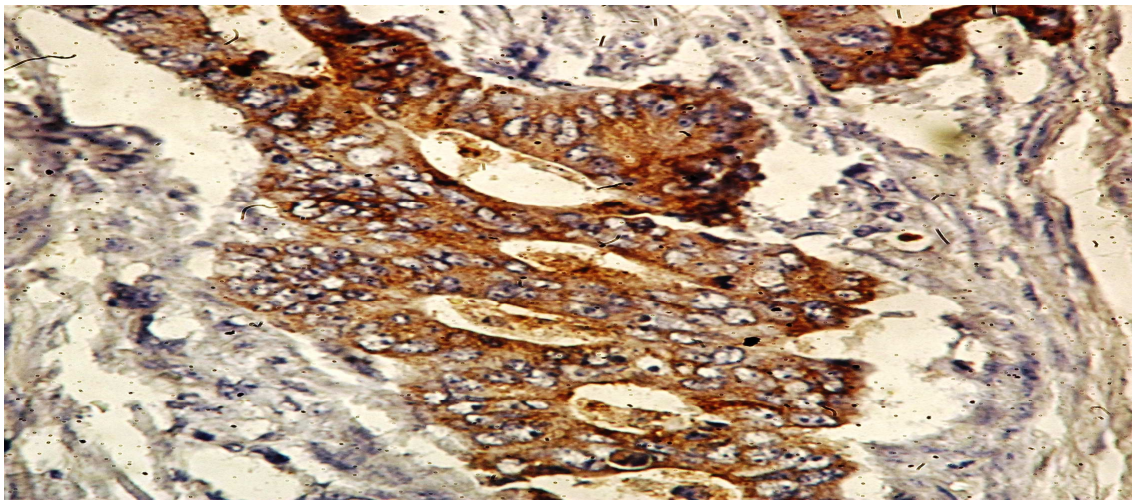

Figure 1:-color colorectal carcinoma : bcl2 staining , weak positive , 40x.

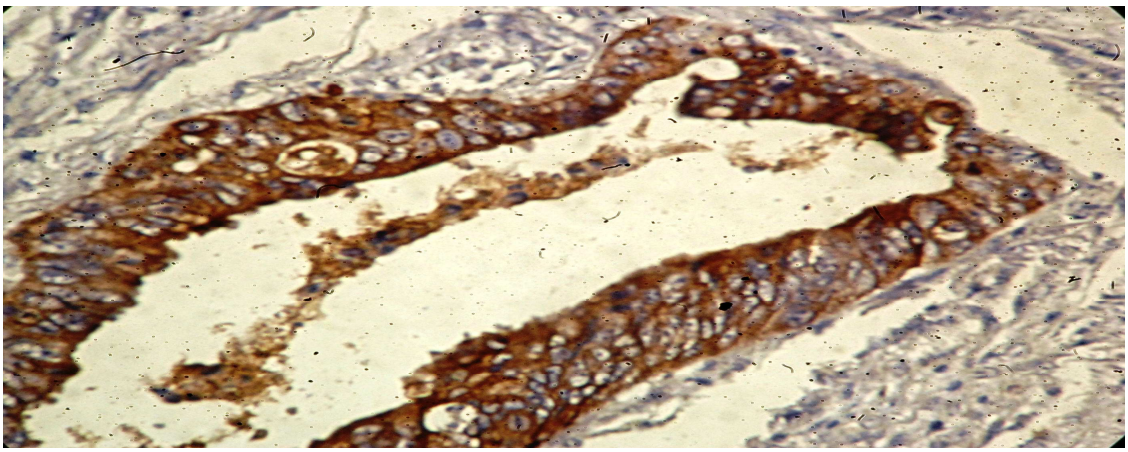

Figure 2:-color colorectal carcinoma : bcl2 staining , moderate positive , 40x.

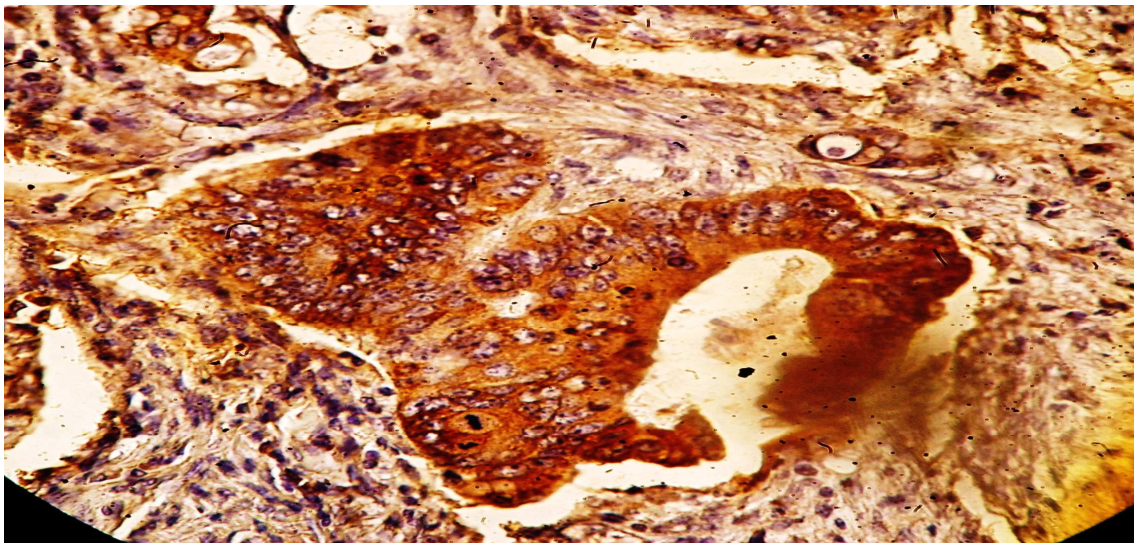

Figure 3:- color colorectal carcinoma : bcl2 staining , strong positive , 40x.

Discussion:-

Immunohistochemical phenotyping of tumors may provide Important information concerning tumor behavior. Although The known function of bcl-2 indicates a possible prognostic role for this oncoprotein.

Our study patient age ranges from(35-90) years with a mean of 67.8 which similar to Ofner D et al 1995. There was no statistical significant relationship between age and sex of patients and expression of bcl2 oncoprotien in colorectal carcinoma; this agreement with Ofner D et al 1995(32), Miao Ouyang et al 2005 (33) .

According to site of colorectal carcinoma in our study show high number of rectal carcinoma showed positive expression(38.5%) more than in colonic carcinoma (16.7%) but no statistically significant between site of tumor and bcl2 expression , this result agreement with Petrisor O et al 2008 (34) , in their study the positive expression of bcl2 in rectal was (60%) more than colonic (46.6%) from the 15 cases of colonic ,and 15 rectal carcinoma with no significant association with any of two locations of rectal and colonic carcinoma, the high percentage of expression more than our study referred to high number of grade III that taken in our study.

According to grade of colorectal carcinoma, in our result bcl2 expression is more in well differentiated_moderated differentiated carcinoma more than the expression of bcl2 in poor differentiated, there is a significant association between bcl2 expression and grade of tumor, so the role of bcl2 in colorectal carcinoma is believed to being a favorable prognostic factor, these result similar to Miao Ouyang et al 2005 were (79.3%) of cases with strong positive in grade I, (60.9%) of cases with moderate positive in grade II(33).

According to stage of tumor ,in our study showed strong positive is more in stage A and stage B, than in stage C ,and negative in stage D, with a significant correlation between stage of tumor and high expression of bcl2 this result agree with Petrisor O et al 2008 (34) where expression of bcl2 was significantly higher in stage A,B than that in C,D (90%, 77.8% versus 60%, 57.2%) the high % in comparison to our study because high number of stage C,D were give negative. Also our result agree with Petrisor O et al 2008 (34) while disagree with Ofner et al (32) where strong positive was higher in stage A and stage B than in stage C and stage D with no significant relation between stage of tumor and bcl2 expression.

Conclusion:-

Bcl2 oncoprotien expression in colorectal carcinoma has been demonstrated as abeing a favorable prognostic factor and associated with less aggressive tumor behavior and or may reflect different stages of tumor progression. However, further research is necessary with include large number of sample and correlation with other tumor marker.

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