Assessment of Bcl-2 oncoprotein expression in colorectal epithelial neoplasms An Immunohistochemical study

Zahraa Marwan Shaban; Mohammed Sami Saeed**
** Pathology Department, College of Medicine, University of Mosul*

Abstract

Colorectal cancer is one of the most frequent cancers in the world. Colorectal cancer is the result of genetic and epigenetic alterations that cause disorders in cell growth, differentiation and apoptosis, resulting in transformation of normal epithelium to adenocarcinoma. Expression of Bcl-2 gene is believed to enhance tumor progression by countering apoptosis triggers, resist DNA damage-induced apoptosis, and undergo growth arrest in G0/G1 or G2/M phases, which promotes tumor cell survival and oncogenic progressions, and may inhibit cell growth and proliferation. To evaluate Bcl-2 oncoprotein expression in colorectal epithelial neoplasms, to correlate the Bcl-2 immunopositivity with certain clinicopathological parameters and to compare the results with other studies. This is a cross sectional study of 50 cases of colorectal adenocarcinoma and 10 samples of colorectal adenomatous polyps. The samples were collected from Mosul Hospitals and some private lab. Patient's age and sex in addition to the site of the tumor was obtained from the archival histological reports. Hematoxylin and Eosin stained section from each case were revised regarding the histological type, grade and stage of the tumor. Immunohistochemical stain for Bcl-2 oncoprotein was performed. The staining assessment of Bcl-2 was done by using a semi-quantitative immunostaining score (ISS) for Bcl-2, taking both intensity and the percentage of stained cells to obtain an IS score that ranged from 0 to 4.0**.** The mean age of all cases was 53.8 years The Fifth decade takes the peak incidence, followed by the seventh decade. Male to female ratio was 1.4:1. Microscopically, 45 (90%) of cases were classical adenocarcinoma, 2 (4%) were mucinous adenocarcinoma, 2 (4%) were signet ring carcinoma and 1 (2%) was neuroendocrine carcinoma. Eight (16%) were well differentiated classical adenocarcinoma, 22(44%) were moderately differentiated, and 15(30%) were poorly differentiated tumor. Duke's staging, 1(2%) case was Duke's A, 18(36%) cases were Duke's B, 25(50%) cases were Duke's C and 6(12%) cases were Duke's D. The Bcl-2 Positivity was observed in 38% of colorectal carcinoma. No significant correlation identified between Bcl-2 expression and the patient's age and sex despite the finding of predominance of Bcl-2 immunoreactivity in young age group and in female. Bcl-2 positivity correlates significantly with the macroscopic type of the tumor with highest positivity in a polypoidal tumor. Bcl-2 expression seen only in colorectal adenocarcinoma classical (NOS) type, while it was negative in other types (p=0.045). An inverse significant correlation between Bcl-2 expression and histological grading was reported where Bcl-2 was reported mainly in well and moderately differentiated tumors (42.1 % and 52.7% respectively). No significance correlation identified between Bcl-2 expression and tumor Duke's stage.

Conclusion: Bcl-2 expression was found in 38% of cases. It was significantly correlated with tumor macroscopic types, histological types and grade. No correlation found between Bcl-2 and age, sex, site and stage of the tumors.

Key Words: Colorectal cancer, Bcl-2 expression, immunopositivity

Introduction

Colorectal cancer is one of the most frequent cancers in the world with a very high mortality rate[1]. It is the third most common cancer in men and the second in women worldwide[2]. It accounting for 8% of all cancer deaths, making it the fourth most common cause of death from cancer[3]. Incidence rates are substantially higher in men than in women (overall sex ratio is 1.4:1)[3]. The peak incidence for colorectal cancer is 60 to 70 years of age; fewer than 20% of cases occur before the age of 50 years[4].
Colorectal cancer is the result of genetic and epigenetic alterations that cause disorders in cell growth, differentiation and apoptosis, resulting in transformation of normal epithelium to adenocarcinoma[5]. Increase cell proliferation and decrease in apoptosis causing colorectal carcinoma, indicating that colorectal carcinogenesis might arise through the disruption of the balance of apoptotic and proliferative mechanisms[6]. One of the potential biomarker for the control of this balance is Bcl-2 oncoprotien[6].
Bcl-2 is the acronym for the B-cell lymphoma/leukemia-gene. Bcl-2 protein is a 26 kD intracellular membrane-associated protein[7].The Bcl-2 protonocogene is localized to the nuclear envelope, endoplasmic reticulum and mitochondrial membranes of hematopoietic and lymphoid cells, a variety of complex differentiating epithelium[8,9] .
Expression of Bcl-2 gene is believed to enhance tumor progression by countering apoptosis triggers[10]. Tumor cells overexpressing Bcl-2, resist DNA damage-induced apoptosis and undergo growth arrest in G0/G1 or G2/M phases, which promotes tumor cell survival and oncogenic progressions but does not enhance cell proliferation and even may inhibit cell growth and proliferation [11,12].

The aims of this study are to evaluate Bcl-2 oncoprotein expression in colorectal epithelial neoplasms, to correlate the Bcl-2 immunopositivity with certain clinicopathological parameters and to compare the results with other studies.

Patients and methods

This is a cross sectional study of 50 cases of colorectal adenocarcinoma and 10 samples of colorectal adenomatous polyps. The samples were collected from Mosul Hospitals and some private lab during a period extended from January 2011 until January 2012. Patient's age and sex in addition to the site and size of the tumor were obtained from the archival histological reports. Hematoxylin and Eosin stained section from each case were revised regarding the type, grade and stage of the tumor. The cases of adenocarcinoma were staged according to Dukes' staging system.

Immunohistochemical analysis:
Sections were cut at 4 µm thicknesses from representative paraffin blocks, mounted onto Sakura White Adhesion Slides (code 9594) and let to dry at 55-60°C in the incubator overnight. Sections then dewaxed and rehydrated by descending grades (100-90%) of ethanol to distilled water. Antigen retrieval (Tris/EDTA buffer, pH 9. code 2368) was achieved by heat-treat at 95-99°C in water bath for 40 minutes, then left to cool at room temperature. Slides were quenched with peroxidase blocker for 10 minutes, followed by washing with Tris-buffered saline (TBS) (Dako code S3006). Slides were incubated with 1:50 diluted primary antibody (Monoclonal Mouse Anti-Human bcl-2 Oncoprotein Clone: 124. Isotype: IgG1Kappa. Dako) for 40 minutes at room temperature in a moisture chamber, then rinsed with TBS for 5 minutes. After which followed by application of secondary

antibody (EnVision + System-HRP (AEC). code K4004. Dako) for 30 minutes and then washed by TBS and distilled water for 5 minutes, remove excess of TBS and followed by adding AEC+ substrate-chromogen solution for 10 minutes to produce the characteristic brownish-red cytoplasmic staining. A counter stain of hemotoxylin for 15 seconds was used to give better view. The final stages were drying and mounting coverslides. For each run of staining a positive and negative control slides were also prepared. A positive control was reactive tonsil and internal mucosal lymphocytes. The negative controls were prepared from a known case of Bcl-2 positive block with substitution of primary antibody by a negative control (Dako Mouse IgG1, (code X0931)).

The staining assessment of Bcl-2 was done by using a semiquantitative immunostaining score (ISS) for Bcl-2, which took both the intensity of staining and the percentage of stained cells to obtain an IS score that ranged from 0 to 4.0 [13, 14]. The intensity score ranges from (0-4). While the other scale was the percentage or the proportion of cells stained at each intensity ranging from (0-100%), then the percent of cells multiplied by the corresponding intensity value to obtain an ISS that ranged from 0 to 4. A score equal or more than 0.5 consider as positive.

Statistical analysis:

A chi square (X^2) or Fisher Freeman Haltom test was used to test for correlations. Statistical significance was considered achieved when the p-value was less than or equal to 0.05 [15].

Results

During the period of 12 months, 60 cases of colorectal epithelial neoplasms were collected. From those, 50 cases were primary colorectal cancer and 10 cases were adenomas.

Colorectal cancer, clinical findings:

The patients' age range from 29 to 85 years (mean 53.8 years), most of them were in the fifth decade (17/50, 34%) and seventh decade (14/50, 28%), figure (1). There was 29 (58%) male and 21(42%) female.

Colorectal cancer, histological findings:

There was predominance of colonic tumors 28(56%), while 22(44%) were rectal tumor. Macroscopically, 17(34%) cases were polypoidal, 16(32%) cases were infiltrative and 17 (34%) cases were ulcerative. Microscopically, 45(90%) cases were of classical adenocarcinoma, 2(4%) cases were mucinous adenocarcinoma, 2(4%) cases were signet ring carcinoma and 1 (2%) case was neuroendocrine carcinoma. The classical adenocarcinoma cases were graded into well differentiated 8(16%) cases, moderate differentiated 22(44%) cases, and poorly differentiated 15(30%) cases. About staging, 1(2%) case was Duke's A, 18(36%) cases were Duke's B, 25(50%) cases were Duke's C and 6(12%) cases were Duke's D.

Colorectal cancer; Bcl-2 status:

The Bcl-2 Positivity was seen in the cytoplasm of malignant colorectal glands. It was observed in 19 out of 50 cases (38%) colorectal carcinoma.

Bcl-2 expression and some clinicopathological parameters:

There was no significant correlation identified between Bcl-2 expression and the patient's age (p=0.791), table (1). Bcl-2 positivity was more in female, but it failed to shows a significant relation with the sex (p=0.075), table (2). Bcl-2 expression was statistically insignificance in correlation with the tumor site (p=0.166), although Bcl-2 was positive in 68.4% of colonic tumor compared to 31.6% in rectum, table (3).

In correlation to the macroscopic types of the tumor, Bcl-2 positivity correlates significantly with the type of the tumor (p=0.003), with a highest positivity seen in a polypoidal tumor, table (4). Histologically, Bcl-2 expression statistically had significant correlation with tumor types (p=0.045) in which Bcl-2 positivity seen only in colorectal adenocarcinoma classical (NOS) type, while it was negative in other types, including classical adenocarcinoma with mucinous component, table (5). Regarding Histological grading: There was an inverse highly significant correlation between Bcl-2 expression and histological grading. The Bcl-2 positivity differs in well and moderately differentiated tumors (42.1 % and 52.7% respectively) from poorly differentiated tumors (5.2%) with a p value equal 0.001, table (6). Bcl-2 expressed statistically insignificant in correlation with the stage of the tumor (p=0.069). Although the highest Bcl-2 immunopositivity was in stage B followed by stage C, table (7)

Colorectal adenomatous polyps:

Ten cases of colorectal adenomas were evaluated, the age of the patients range from 19-75 years. The male to female ratio was 1:1. Seven cases were from the colon and the rest were from the rectum. Sixty percent were tubullovillous type, 30% tubular type and 10 % villous type. Twenty percent showed mild dysplasia, 30% showed moderate dysplasia and 50% harboring severe dysplasia. Bcl-2 positivity seen in 5 (50%) of all adenomas. Two (40%) of cases were tubular type, 2 (40 %) were tubullovillous and 1 (20%) was villous adenoma. However all these positive cases were barbering severe dysplasia.

Discussion

Colorectal carcinoma accounts for about 10 % of all body cancers [16]. It is the second leading cause of death from malignancy in the industrialized world [16]. However, in developing counties mainly in Asian countries like, China, Japan, South Korea and Singapore, economic development and the adoption of earlier Western life-styles have led to an increased colorectal cancer incidence similar to that of developed countries [16,17].

Colorectal cancers have been reported to show bcl-2 overexpression and it appears that the role of bcl-2 is probably more important in the early development of colorectal tumors than in later tumor progression as it may lacks with loss of tumor differentiation, local invasion, metastasis and recurrence of colorectal cancers [18-19].

In the current study bcl-2 was expressed in 38% of the cases. Other similar studies had shown variable ratios ranging from 20.2% to 69.5%, table (8).

The fourth and seventh decades were the most common age group affected by colorectal cancer. However; there was no significance correlation between the age and the Bcl-2 expression, this is consistent with all other studies(7,28,29,31,32,33,34).

In this study there was male predominance with male to female ratio of 1.4:1. But despite that Bcl-2 expression reported more in female. However; no significant correlation was found with sex al. Our results is consistent with other studies which had reported also no significant correlation with sex[28,29,31,34,35]. But a single study found a significant correlation with a female predominant[7]. This could be accidental or explained by the expression of Bcl-2 protein among tissues with direct hormonal influence[7].

Bcl-2 expression in our study was not significant weather the tumor located in the proximal or distal colon, this agree with many studies[7,23,30,31,33,34,36].

A significant correlation between Bcl-2 expression and the polypoidal, fungating, colorectal tumors was detected in our study. This is consistent with two other studies which suggested that the Bcl-2 expression became significantly lower as the tumors became flatter or took on a depressed form[21,37]. On the other hand single study showed no significant correlation with the macroscopical types of colorectal cancer[15].

Bcl-2 positivity identified only in adenocarcinoma classical type (100%), whereas it was negative in all other types of colonic carcinoma in this current study. Accordingly, there is a significant correlation of Bcl-2 expression and the tumor histological types (p= 0.045). Pinar et al and Manne et al compared between classical adenocarcinoma and mucinous carcinoma and found a significant relation to tumor types[33,38]. Other studies showed no significant correlation with the histological types of colorectal cancers[20,30,39].

A significant inverse relationship between expression of Bcl-2 oncoprotien and grading of tumor was reported in our study. Similar association was noticed by Leahly et al[34]. Many studies had shown that Bcl-2 expression more evident in well and moderate differentiated tumors than poorly, but failed to reach the significant level[7,21,27,40].

The majority of Bcl-2 positivity was in stages B and C (94.8%), but despite of this result, the relation between Bcl-2 and stage of tumor failed to reach the significant level (p=0.069). This may be explained by the low number of our cases in stage A. This agree with many studies, that showed no significant correlation between Bcl-2 expression and tumor stage[7,20,21,26,27,34,39]. This result suggested that Bcl-2 have a role in early phase of colorectal carcinogenesis.

Regarding of Bcl-2 expression and colorectal adenomas: half of the cases (50%) studied show Bcl-2 positivity and all of these cases were having severe dysplasia. Bosari et al, stated that Bcl-2 immtmoreactivity was noted in all adenomas irrespective of the degree of dysplastic change; it was diffuse in 84% of adenomas and focal in the remaining cases[8]. Saleh et al, found about 78.6 % of adenomas showed Bcl-2 positivity[41]. In these studies Bcl-2 expression was highly in colorectal adenomas than carcinoma and this agree with the fact that Bcl-2 oncoprotein expression is probably an early step in the process of colon carcinogenesis, and its expression may be associated with favorable pathological parameter[41].

Several studies assessed the hypothesis that Bcl-2 expression in colorectal cancer is associated with a good clinical outcome and considered as a good prognostic factor this is obvious from the association of Bcl-2 immunoreactivity with the well and moderately differentiated colorectal adenocarcinoma [34], which explanted by the role of Bcl-2 in down cell growth of colorectal cancer [34]. Therefore, loss of Bcl-2 may be indicative of a more aggressive phenotype allowing further tumor progression under influences such as p53 changes [34].

Conclusions:

Bcl-2 expression was found in 38% of cases. It was significantly correlated with tumor macroscopic types, histological types and grade. No correlation found between Bcl-2 and age, sex, site and stage of the tumors.

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Table (1): bcl-2 expression and patient's age.

Age/ years	Bcl-2 positive		Bcl-2 negative		Total		P-Value
	No.	%	No.	%	No.	%	
≤40	3	15.8	8	25.8	11	24.0	0.791 NS)(
41-50	7	36.9	10	32.3	17	23.0	
51-60	2	10.5	1	3.2	3	6.0	
61-70	5	26.3	9	29.0	14	30.0	
>71	2	10.5	3	9.7	5	10.0	
Total	19	100	31	100	50	100	

Table (2): bcl-2 expression and sex.

Sex	Bcl-2 positive		Bcl-2 negative		Total		P-Value
	No.	%	No.	%	No.	%	
Male	8	42.1	21	67.8	29	58.0	0.075 NS)(
Female	11	57.9	10	32.2	21	42.0	
Total	19	100	31	100	50	100	

Table (3): bcl-2 expression and the site of the tumor.

Site	Bcl-2 positive		Bcl-2 negative		Total		P-value
	No.	%	No.	%	No.	%	
Colon	13	68.4	15	48.4	28	56.0	0.166 (NS)
Rectum	6	31.6	16	51.6	22	44.0	
Total	19	100	31	100	50	100	

Table (4): bcl-2 expression and the macroscopical types of colorectal carcinoma.

Macroscopical Types	Bcl-2 positive		Bcl-2 negative		Total		P-value
	No.	%	No.	%	No.	%	
Polypoidal	12	63.2	5	16.1	17	34	0.003 (HS)
Ulcerative	3	15.8	14	45.2	17	34	
/annularInfiltrative	4	21.0	12	38.7	16	32	
Total	19	100	31	100	50	100	

Table (5): bcl-2 expression and the histological type of the tumor.

Histological type of the tumor	Bcl-2 positive		Bcl-2 negative		Total		P-value
	No.	%	No.	%	No.	%	
classical adenocarcinoma (NOS)	19	100	26	83.8	45	90	0.045 (HS)
Mucinous adenocarcinoma	0	0	2	6.5	2	4	
Signet ring adenocarcinoma	0	0	2	6.5	1	4	
Neuroendocrine carcinoma	0	0	1	3.2	2	2	
Total	19	100	31	100	50	100	

Table (6): bcl-2 expression and tumor grade.

Grade	Bcl-2 positive		Bcl-2 negative		Total		P-value
	No.	%	No.	%	No.	%	
Well differentiated	8	42.1	0	0.0	8	17.8	0.001 (HS)
Moderate differentiated	10	52.7	12	46.2	22	48.9	
Poor differentiated	1	5.2	14	53.8	15	33.3	
Total	19	100	26	100	45	100	

Table (7): bcl-2 expression and tumor stage.

Stage	Bcl-2 positive		Bcl-2 negative		Total		P-value
	No.	%	No.	%	No.	%	
A	0	0.0	1	3.2	1	2.0	0.069 (NS)
B	10	52.7	8	25.8	18	36.0	
C	8	42.1	17	54,9	25	50.0	
D	1	5.2	5	16.1	6	12.0	
Total	19	100	31	100	50	100	

Table (8): Bcl-2 expression in colorectal cancer in different studies

Study	Region	Year	No. of cases	% of Bcl-2 positivity
Current study	Mosul	2012	50	38%
Sun et al[20]	China	2010	128	69.5
Poincloux et al[21]	France	2009	226	36%
Torsello et al[22]	Italy	2008	1340	48.5%
Petrişor et al[23]	Romania	2008	30	53.3%
Ismail et al[24]	Egypt	2007	104	26%
Bidgoli et al[25]	Iran	2007	54	33.3%
Tsamandas et al[26]	Greece	2007	56	37%
Demirbaş et al[27]	Turkey	2006	124	20.2%
Han et al[28]	Korea	2006	81	39.5%
Dan-ping et al[29]	China	2005	93	57%
Chatla et al[14]	USA	2005	158	56.3%
Watson et al(30)	UK	2005	437	45.5%

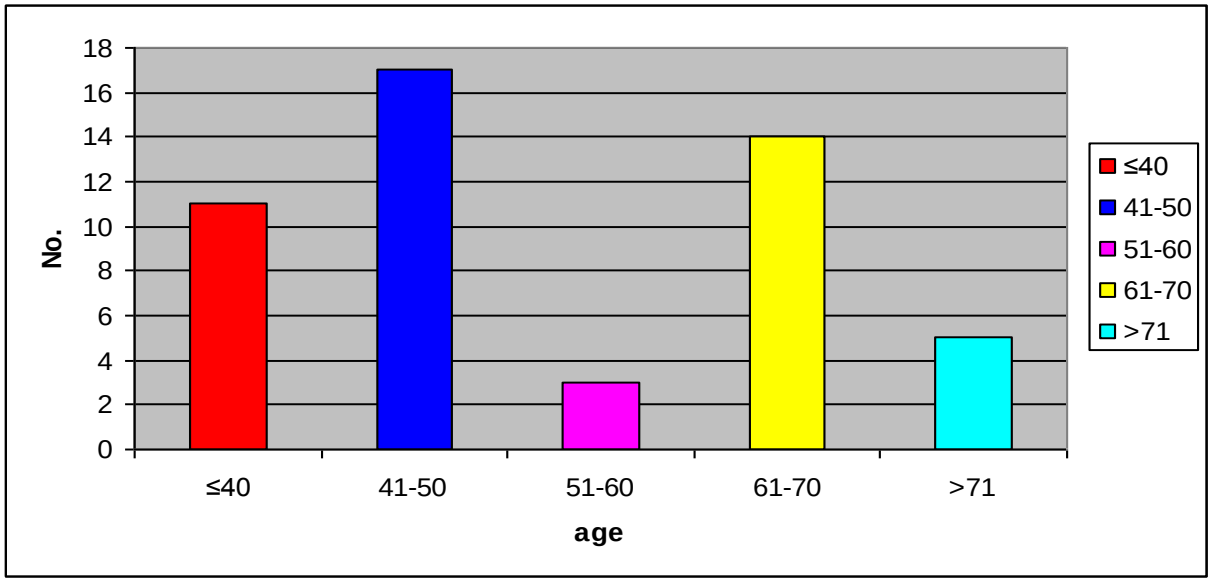

Figure (1), the age distribution of colorectal cancer cases.

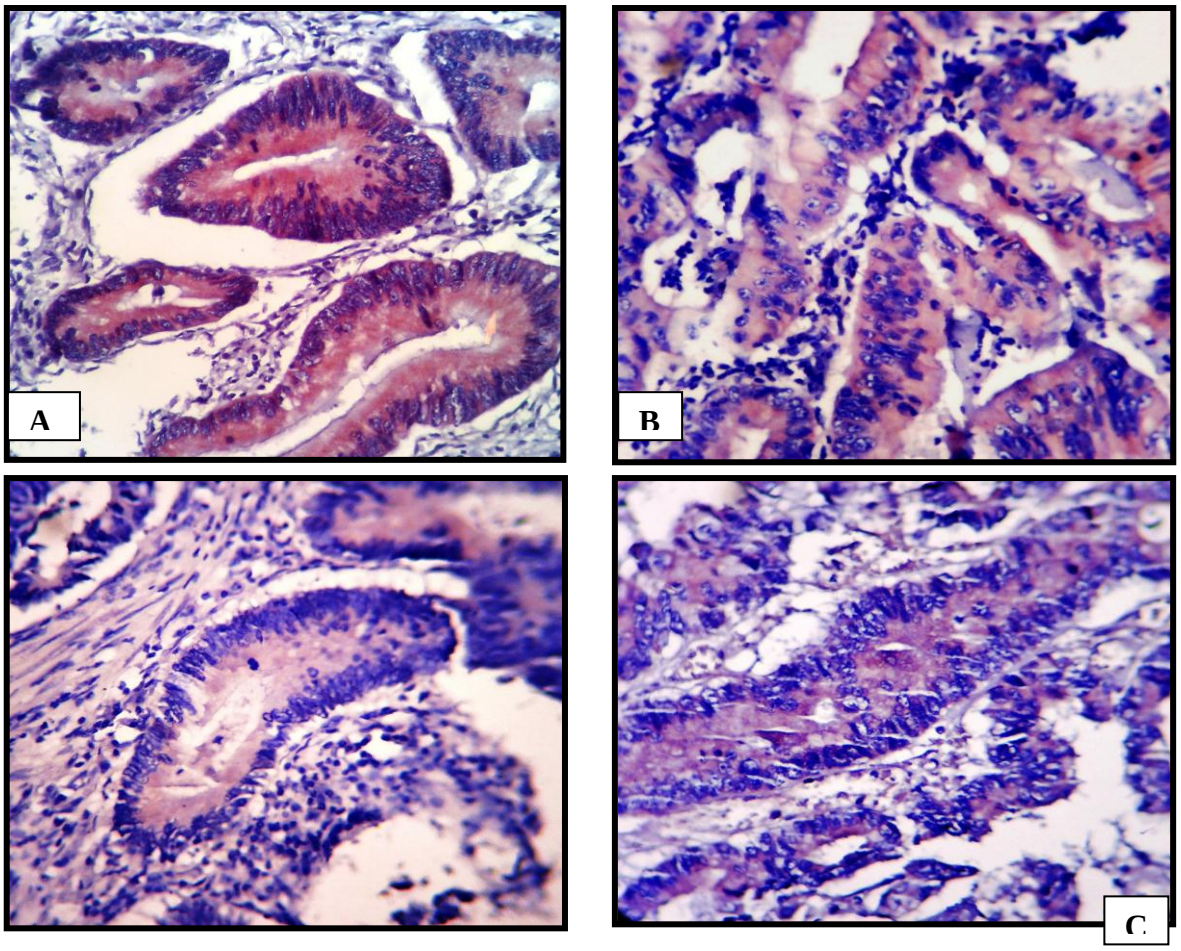

Figure (2): Different intensity of staining, (A. Strong, B. Moderate, C. Mild, D. Weak) (original magnification IHC; x400).

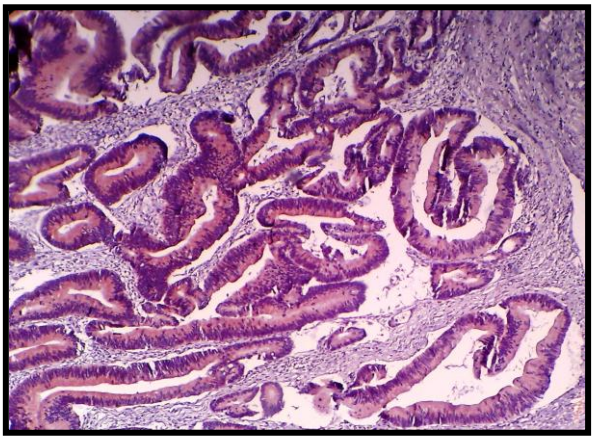

Figure (3): well differentiated adenocarcinoma, (IHC, score 3.6; x100)

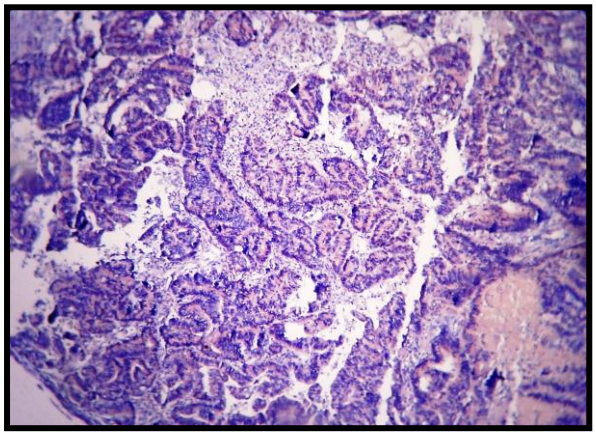

Figure (4): moderate differentiated adenocarcinoma, (IHC, score 2.85; x100)

الخلاصة

خلفية البحث: سرطان القولون والمستقيم هو واحد من أكثر السرطانات شيوعا في العالم .سرطان القولون والمستقيم هو نتيجة لتغيرات جينية التي تسبب اضطرابات في تمايز الخلايا والنمو وموت الخلايا المبرمج، مما أدى إلى التحول من النسيج الظهاري الطبيعي الى سرطانة غدية .ويعتقد أن التعبير عن الجين2-Bcl لتعزيز نمو أورام سرطانية وذلك بالتصدي لمشغلات موت الخلايا المبرمج، ومقاومة الحمض النووي من التلف الناجم عن موت الخلايا المبرمج، والخضوع لتوقيف نمو في G0/G1أو مراحلM / G2 ، وهو ما يعزز بقاء الخلايا السرطانية وتعاقب تكون الورم، و قد تمنع نمو الخلايا وانتشارها.
الأهداف: لتقييم تعبير البروتين السرطاني Bcl-2 في الأورام الظهارية (الطلائية) للقولون والمستقيم،ولربط التفاعلية المناعية ل Bcl-2 مع معلمات سريرية مرضية معينة، ومقارنة النتائج مع نتائج الدراسات الأخرى.
الطرق والأدوات: هذه الدراسة مقطعية ل ٥٠ حالة من السرطانة الغدية للقولون والمستقيم و ١٠ عينات من الاورام الحميدة الغدية للقولون والمستقيم .تم جمع العينات من مستشفيات الموصل وبعض المختبرات الخاصة .وقد تم الحصول على عمر المريض والجنس، بالإضافة إلى موقع وحجم الورم من التقارير النسيجية الأرشيفية .تم تنقيح هيماتوكسيلين وإيوزين القسم الملون من كل حالة على حدة فيما يتعلق بنوع النسيجية، درجة ومرحلة الورم .وأجريت صبغة مناعية نسيجية كيميائية للبروتين السرطاني Bcl-2 .وقد تم تقييم اصطباغ Bcl-2 باستخدام درجة المناعية شبه كمي (اي اس اس) لBcl-2، مع كل شدة، والنسبة المئوية للخلايا الملون للحصول على درجة هو الذي تراوح من ٠ إلى ٤.٠.
النتائج: وكان متوسط العمر لجميع الحالات٥٣,٨ سنوات .العقد الخامس يأخذ أعلى نسبة ارتفاع، تليها العقد السابع .وكانت نسبة الذكور إلى الإناث ١.٤:١ .ومجهريا كانت٤٥(٩٠%)من الحالات غدية الكلاسيكية، وكانت٢ (٤%) سرطانة غدية مخاطينية (موسيني)،و٢ (٤%) سرطان الخاتم الدائري و١(٢٪) سرطان الغدد الصم العصبية . وكانت ثمانية (١٦%) متميزة جيدة من سرطان الغدية الكلاسيكية، و٢٢ (٤٤%) ذات تميز متوسط ، و ١٥ (٣٠%) كانت ذات تميز ضعيف . وبالنسبة لمرحلة تصنيف ديوك،١(٢%) حالة; كانت طور، ديوك أ وكان ١٨ (٣٦%) من الحالات كانت ديوك ب، ٢٥ (٥٠%) من الحالات كانت طور ديوك س و ٦ (١٢%) من الحالات كانت ديوك د. وقد لوحظ فيBcl-2 إيجابية في ٣٨% من سرطان القولون والمستقيم . ولا يوجد ترابط كبير بين تحديد تعبير Bcl-2 وعمر المريض ونوع الجنس على الرغم من العثور على غلبة تفاعلية مناعية لBcl-2 في الفئة العمرية من الشباب والنساء. وقد لوحظ ان ايجابية Bcl-2 ترتبط بشكل كبير مع نوع العيانية المرئية للورم مع أعلى إيجابية ل Bcl-2 في الورم السليلاني. وقد لوحظ ايضا ان تعبير Bcl-2 ينظر فقط في اورام القولون والمستقيم نوع غدية الكلاسيكية، في حين كانت سلبية في أنواع أخرى (P = 0.045) . وكذلك لوحظ هنالك إرتباطية عكسية ذات دلالة إحصائية بين تعبير Bcl-2 والمرحلة النسيجية للورم او الصف وخاصة في الاورام ذوات التميز الجيد والتميز المتوسط (٤٢,١% و ٥٢.٧% حسب التسلسل). وكذلك لوحظ عدم وجود علاقة احصائية بين تعبير -Bcl 2 وأطوار او مراحل ديوك .
الاستنتاج: تم العثور على تعبير البروتين السرطاني Bcl-2 في ٣٨% من الحالات. وقد ارتبط بشكل كبير مع انواع العيانية المرئية للورم وأنواع النسيجية والصف . ولايوجد ترابط بينه وبين العمر،الجنس،موقع ومرحلة الورم السرطاني.