Bcl-2 expression and image analysis of premalignant and malignant colonic lesions

Sonia L. El-Sharkawy, Naglaa F. Abbas, Wafaa E. Abdelaal, Manal A. Badawi

Department of Pathology, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt

Correspondence to Manal A. Badawi, MD, Department of Pathology, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Postal Code: 12622, Egypt. Tel.(Office): 0233371615 (1214); e-mail: badawimanal@yahoo.co.uk

Received: 7 September 2023
Revised: 20 November 2023
Accepted: 3 December 2023
Published: 10 June 2024

Journal of The Arab Society for Medical Research 2024, 19:63–72

Background/aim
Colorectal cancer is the third malignancy worldwide with high mortality. The development of colorectal carcinoma is a multiple step process that turns normal cells into malignant ones. One of these steps is inhibiting apoptosis. Bcl-2 is the key regulators of apoptosis and thus inhibits programmed cell death. The relationship between cell death and cell proliferation is balanced through apoptosis. This study aimed to evaluate immunohistochemical bcl-2 expression, nuclear morphometric parameters, and cell cycle values in premalignant and malignant colon lesions.

Material and methods
Sixty colonic paraffin blocks (10 normal mucosa, 20 adenomas, and 30 carcinomas) from private laboratories and the Pathology Department, National Research Centre, Cairo, Egypt, were included in this study. Bcl-2 expression was evaluated by immunohistochemistry. Nuclear morphometric parameters and cell cycle values were studied using an image analysis system.

Results
Immunohistochemical results showed expression of bcl-2 in the lower half of normal colonic crypts. Bcl-2 positivity was detected in 53% of carcinomas and 85% of adenomas with significant difference. The percentage of bcl-2 positive cells in carcinomas was significantly decreased with increasing grades. In carcinomas, nuclear area showed significant increase with increasing grades. Nuclear area showed significant difference between high-grade dysplastic adenomas and carcinomas. Carcinomas showed high proportion of aneuploid cells with significant difference than adenomas. Inverse correlation was detected between aneuploidy and bcl-2 positivity.

Conclusion
Bcl-2 protein has a role in early event of colorectal carcinogenesis. The acceptable reliability of immunohistochemical, nuclear area, and cell cycle analysis may serve as diagnostic and prognostic indicators in benign and malignant colorectal lesions.

Keywords:
Bcl-2, colonic carcinoma, image analysis, immunohistochemistry

J Arab Soc Med Res 19:63–72

1687-4293

Introduction

Colorectal cancer (CRC) is the third malignant tumor with high mortality accounting for about 881.000 deaths worldwide [1,2]. This tumor is the second cancer in women and the third in men [3]. Its incidence in developed countries is increasing among persons less than 50 years old [4]. Limited clinical signs are demonstrated in early stages of CRC and patients are diagnosed with metastasis, rendering difficult therapy, therefore early diagnosis is a challenge [5,6].

Bcl-2 family of proteins which regulate apoptosis, and disturbance of this regulation can lead to many pathological consequences including formation of malignant tumors [7]. The antiapoptotic bcl-2 gene is 26-kD protein which blocks apoptosis and controls the release of preapoptotic factors. These factors are responsible for stabilizing the mitochondrial membrane and activation of caspase [8].

Aberrant expression of bcl-2 is closely related to carcinogenesis in most malignant tumors, including CRC [8]. Bcl-2 family members are classified into three subgroups: the antiapoptotic/prosurvival proteins, the proapoptotic proteins, and the multidomain proapoptotic proteins [9]. The proapoptotic proteins are characterized by the presence of the N-terminal BH4 domain. This domain can bind other proteins which are not from bcl-2 family proteins, forcing them to play a role in inhibiting apoptosis by functions such as, autophagy, proliferation, DNA repair differentiation, and angiogenesis [10]. The antiapoptotic protein group makes their prosurvival role by binding and inhibiting the proapoptotic protein which are the effectors of apoptosis and the sensors of cellular stress [11].

Aberrant expression of bcl-2 proteins has been detected in malignant tumors from different origins. Other studies have shown that high levels of antiapoptotic proteins have an action in modulating apoptosis, response to therapy, tumor initiation, and progression [10,12]. Several studies detected that bcl-2 expression is associated with cancer progression including colon cancer. Some studies found a correlation between Bcl-2 expression and better prognosis [13,14]. Others demonstrated that bcl-2 was a bad prognostic for patients having malignant tumors [15–17].

Morphometry has a great role in many areas of diagnostic histopathology. Computer-assisted image morphometric analysis has been introduced to quantitate various grading systems. Increasing abnormalities in morphology of nuclei correlates with the advanced grade of tumor as well as poor prognosis in many cancers. Nuclear morphometry correlates with mitotic activity, tumor size, and lymph node metastasis. Nuclear morphometry can be added to clinical and histopathological features and used to prognosticate and classify cancer cases into low-risk and high-risk groups [18].

Cellular growth and cell preparation for division between cell divisions is called the cell cycle. The cell cycle is divided into four phases; the length of cell cycle phases is an important characteristic of cell life. They are the G1 (gap 1), S (DNA synthesis), G2 (gap 2), and M (mitosis) phases [19].

Information about cell cycle is very helpful for predicting tumor development. Cancer cells show unscheduled proliferation, genomic instability, and chromosomal instability [20]. Dysregulation of the cell cycle is the first step in carcinogenesis, tumor invasion, and metastases [21].

The progression of cells in the cell cycle has highly organized steps related to intracellular and extracellular signals and controlled by endogenous and exogenous factors. Various approaches are used to identify cell cycle phases. The most frequently used approach is based on the analysis of DNA. The cells in G1 and G0 have half DNA content as compared to G2 and M cells. The DNA histogram enables the estimation of the percent of cells in G1/G0, S, and G2/M phases [19].

This study aimed to evaluate immunohistochemical expression of bcl-2 and image analysis of nuclear parameters in premalignant and malignant tumors.

Material and methods

Samples and the study design

The present study retrospectively obtained paraffin blocks and reviewed the database of 60 cases that underwent curative surgery for CRC from private laboratoriess and the Pathology Department, National Research Centre. The used material was derived from anonymous archival tissue samples embedded in paraffin blocks. So, no consent was obtained.

The 60 colonic paraffin blocks included in this study were 10 normal mucosa, 20 adenomas, and 30 carcinomas. The adenoma cases were 10 with low-grade dysplasia and 10 with high-grade dysplasia. The carcinoma cases were 10 grade I adenocarcinoma, 10 grade II adenocarcinoma, and 10 grade III adenocarcinoma.

Inclusion criteria

Cases of CRC with no other cancers or chronic diseases and cases did not receive radiotherapy or chemotherapy.

Exclusion criteria

Cases with other cancers or received radiotherapy or chemotherapy and cases with chronic diseases were excluded from this study.

Ethical consideration

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study was approved by the Local Ethics Committee of National Research Centre, Cairo, Egypt with approval number 16/308. The used materials were derived from archived tissue samples embedded in paraffin blocks. The personal data of the patients were replaced by numerical codes for privacy and confidentiality.

Methods

Three sections (4 μm thick) were cut from each block. One section was stained with hematoxylin and eosin for histopathologic evaluation and morphometric analysis. The second section was stained with Feulgen stain for measurement of cell cycle parameters. The third section was mounted on a positively charged glass slide for immunohistochemical staining using anti-bcl-2 antibody.

Immunohistochemistry

For immunostaining, the sections were deparaffinized and rehydrated through a graded series of alcohol. Endogenous peroxidase activity was blocked by freshly prepared 0.3% hydrogen peroxide in methanol for 20 min. Then microwave antigen retrieval was used, followed by incubation with bcl-2 antibody. The Ultravision LP polymer system (Lab Vision, Fremont, California, USA) and the chromogen diaminobenzidine were used to amplify and visualize the antigen–antibody complex. The expression of bcl-2 was evaluated in the entire section at a magnification of ×400. Bcl-2 showed cytoplasmic staining. The bcl-2 was determined as the percentage of positively stained cells to the total number of cells. The percentage positivity was graded as low (>25%), medium (25–50%), and high (<50%) [22].

Image analysis

The image analysis was performed at the Pathology Department, National Research Centre, using the Leica Qwin 500 Image analyzer (LEICA Imaging Systems Ltd, Cambridge, UK) which consist of a Leica DM-LB microscope with a JVC color video camera attached to a computer system.

Morphometric analysis

Nuclear morphometric analysis was performed on hematoxylin and eosin-stained slides. A 100–150 nonoverlapping nuclei were evaluated per case by a trained pathologist who was blinded of the histopathological diagnosis. Images were captured at ×400 magnification. Calibration was performed before each measuring session. Nuclei were outlined using the mouse of the computer. These outlines were refined automatically and automatic measurements were made by the software. After measurement, the data were transferred to the Excel sheet for further analysis. Nuclear morphometric parameters analyzed included nuclear area, diameter, perimeter, roundness ($4\pi A/P^2$), texture (amount of local intensity variation in the digitized image of a nuclear profile) and mean grey (the sum of grey values for each pixel within the nucleus/number of pixels measured).

Cell cycle analysis

Two of the most popular cytometric applications are the measurement of cellular DNA content and the analysis of the cell cycle. The cell cycle profile was determined by staining the DNA with a specific dye and measuring its intensity. The Feulgen stain is not only specific for the localization of DNA in chromosomes, but at the same time the intensity of the reaction may be considered as an index of the amount of DNA present in the cell [23].

Analysis was performed first on the normal control specimens to determine the reference values. Selection of nuclear boundaries is usually performed automatically by the image analysis system; however, some degree of interaction or editing is usually needed for optimal nuclear selection 'Touching' nuclei can be 'Cut' from each other, and cellular fragments or extraneous cells can be erased prior to measurements. Only separate, intact nuclei were measured. Distorted or overlapping nuclei and nuclear fragments were manually eliminated from measurement. All these facilities were supplied as editing functions in the Leica Qwin 500 image analysis systems.

Care was taken to measure various nuclei representative of the examined lesion, so that measurements were not biased toward the degenerated, bizarre, or anaplastic nuclei. Each field was focused before the measurement to exclude cut nuclei and blurred ones. The optical density of the selected nuclei in each microscopic field is then measured and automatically converted by the system into cell phase in the life cycle. We select many fields until the desired number of nuclei 100–150 has been measured. Percentages of cells in the first growth phase (G1), proliferating cells (S), and second growth phase (G2) were calculated and determined automatically by the system. All collected data were stored to be reanalyzed. The proliferating cell percentage was calculated by summation of percentage of cells in synthesis phase (S) and cells in gap 2 (G2) phase of the cell cycle.

Statistical analysis

All data were collected, revised, coded, and entered into the Statistical Package for the Social Sciences (version 23.1; IBM SPSS, Armonk, New York, USA). The quantitative data were presented as mean±SD. The comparison between groups regarding quantitative data was done using the analysis of variance test. The qualitative variables were presented as number and percentages. The comparison between groups regarding qualitative data was done using the χ^2 test. P values of less than 0.05 were considered to indicate statistical significance.

Results

Bcl-2 immunoreactivity

Normal colonic mucosa showed bcl-2 immunoreactivity in the lower half of colonic crypts.

The epithelial cells lining the upper half of the crypt and surface epithelium were negatively stained for bcl-2. Out of 20 adenoma cases, 17 (85%) cases showed positive staining for bcl-2. Nonsignificant correlation was detected between bcl2 expression and degree of dysplasia in adenomas where 90% of low-grade dysplasia and 80% of high-grade dysplasia were positively stained for bcl-2 (Table 1).

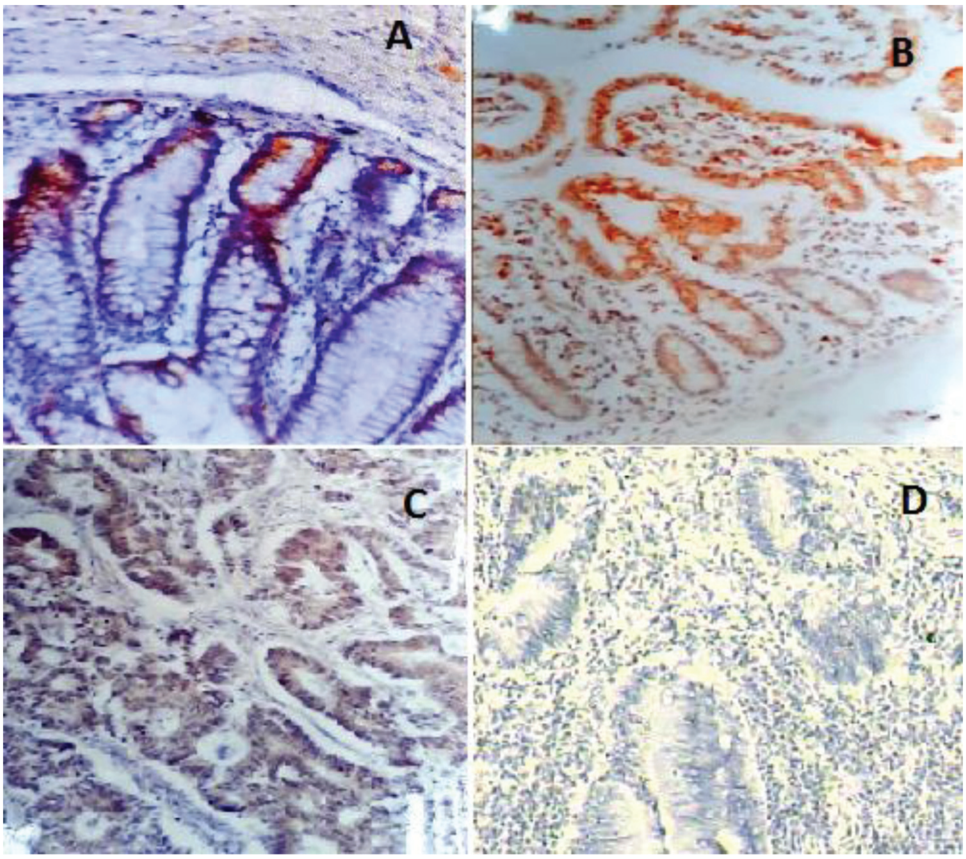
The carcinoma cases had a significantly lower rate of bcl-2 positivity than adenomatous cases where it was detected in only 53.3% of cases (vs. 85% of adenoma cases). Bcl-2 was expressed in 80% of grade I, 50% of grade II, and 30% of grade III showing significant negative correlation between bcl-2 expression and tumor differentiation. Semiquantitative determination of the percentage of bcl-2 immunoreactive tumor cells revealed a significant difference between colonic adenomas and carcinoma where most of the positive adenoma (65%) showed high staining (<50%), while majority of the positive carcinoma (68.9%) displayed low immunoreactivity for

Table 1 Bcl-2 expression in studied groups

	Bcl-2 +ve [*n* (%)]	Bcl-2 −ve [*n* (%)]	Total
Adenomas			
Low grade dysplasia	9 (90)	1 (10)	10
High grade dysplasia	8 (80)	2 (20)	10
Total	17 (85)	3 (15)	20
Adenocarcinoma*			
Grade I	8 (80)	2 (20)	10
Grade II	5(50)	5 (50)	10
Grade III	3 (30)	7 (70)	10
Total	16 (53.3)	14 (46.7)	30

*Significant difference at P value less than 0.05 using the χ^2 test.

Figure 1



Bcl-2 imunohistochemical expression; (a) normal colonic mucosa revealing positive expressin in lower half of colonic crypts (×200), (b) colonic adenoma showing high percetage of positively stained cells (×100), (c) grade I colonic adenocarcinoma showing moderate expression (×100), and (d) grade II colonic adenocarcinoma revealing negative expression (×200).

bcl-2 (>25%). Nonsignificant correlation was detected between the degree of dysplastic changes in adenoma and the proportion of positively stained cells. On contrary a significant inverse correlation was detected between the proportion of bcl-2 positively stained cell and histologic grades of carcinoma on comparing grade I and grade II with grade III. All cases of grade III and only 61.9 of grade I and grade II cases showed low staining. None of grade III cases and 15.5% of grade I and grade II cases showed high staining, as shown in Fig. 1 (Table 2).

Nuclear morphometry

Adenoma cases revealed significantly higher values of nuclear area than those of normal colonic mucosa ($P>0.05$). All nuclear morphometric parameters showed insignificant difference between low-grade and high-grade dysplasia ($P<0.05$). In cases of adenocarcinoma, nuclear area showed significant increase with increasing histologic grade ($P>0.05$), as shown in Fig. 2 (Table 3).

Cell cycle analysis

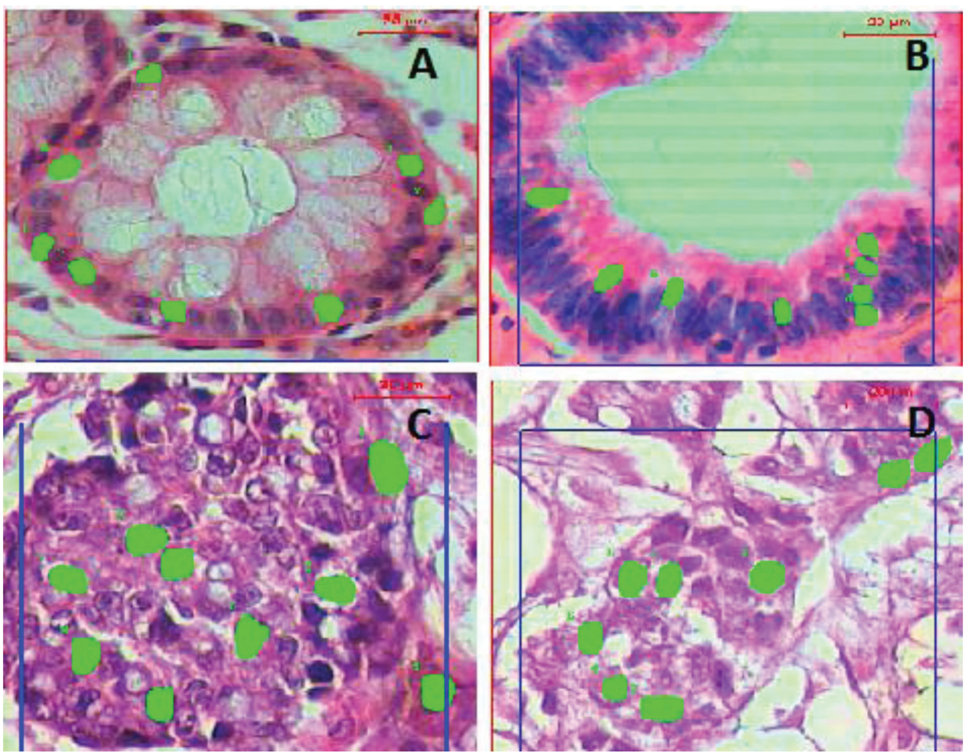
Table 4 showed that the examined cells in the gap 1 (G1) phase significantly decrease from control cases, low grade dysplasia, high grade dysplasia to carcinoma cases with higher grades ($P>0.05$). The percentage of proliferating cells increased significantly in adenomas than control. However, carcinomas showed decreased number of proliferating cells than adenomas. As regard

Table 2 Proportion of bcl-2 positively stained cells in studied groups

	Low >25% [*n* (%)]	Moderate 25–50% [*n* (%)]	High <50% [*n* (%)]	Total
Adenomas				
Low-grade dysplasia	1 (11.1)	2 (22.2)	6 (66.7)	9
High-grade dysplasia	1 (12.5)	2 (25)	5 (62.5)	8
Total	2 (10)	4 (25)	11 (65)	17
Adenocarcinoma*				
Grade I	4 (57.2)	2 (28.5)	1 (14.3)	7
Grade II	4 (66.6)	1 (16.7)	1 (16.7)	6
Grade III	3 (100)	0	0	3
Total	11 (68.9)	3 (18.8)	2 (12.3)	16

*Significant difference at *P* value less than 0.05 using the χ^2 test.

Figure 2



Nuclear morphometry: (a) normal colonic mucosa, (b) adenoma with dysplasia, (c) grade I adenocarcinoma, and (d) grade III adenocarcinoma (hematoxylin and eosin, ×400).

cells in S phase and gap 2 (G2), there was insignificant difference between the studied groups ($P<0.05$). There was a significant increase of the percent of aneuploid cells between studied groups where they increase from low grade dysplasia to high grade dysplasia to carcinoma cases with increasing grades ($P>0.05$) (Fig. 3).

Correlation between Bcl-2 expression and nuclear parameters

There was nonsignificant correlation between nuclear area and proportion of bcl-2 positive cells in cases of adenomas with low-grade and high-grade dysplasia. In carcinoma cases, there was a significant converse correlation between nuclear area and proportion of bcl-2 positive cells with different histologic grades.

Table 3 Nuclear morphometric parameters of the studied cases

	Area (μm^2)	Diameter (μm)	Perimeter (μm)	Roundness	Texture	Mean grey
Normal colonic mucosa	12.9±1.5^a	5.1±0.2^a	14.3±0.7^a	1.23±0.06^a	48.1±7.1^a	234.8±1.2^a
Adenomas						
Low-grade dysplasia	18.5±2.2^b	6.0±0.4^a	17.0±1.2^a	1.20±0.02^a	118.2±21.7^a	219.0±6.2^a
High-grade dysplasia	20.4±1.3^b	6.4±0.1^a	17.9±0.5^a	1.20±0.02^a	69.6±30.9^a	231.5±7.9^a
Adenocarcinoma						
Grade I	23.7±2.1^c	6.8±0.4^a	19.2±0.9^a	1.19±0.01^a	62.8±19.4^a	233.6±6.1^a
Grade II	29.9±0.7^d	7.5±0.0^a	21.4±0.2^a	1.18±0.03^a	62.7±22.2^a	232.5±8.9^a
Grade III	35.2±3.2^e	8.2±0.5^a	23.2±1.3^a	1.17±0.005^a	101.1±13.6^a	230.4±6.8^a

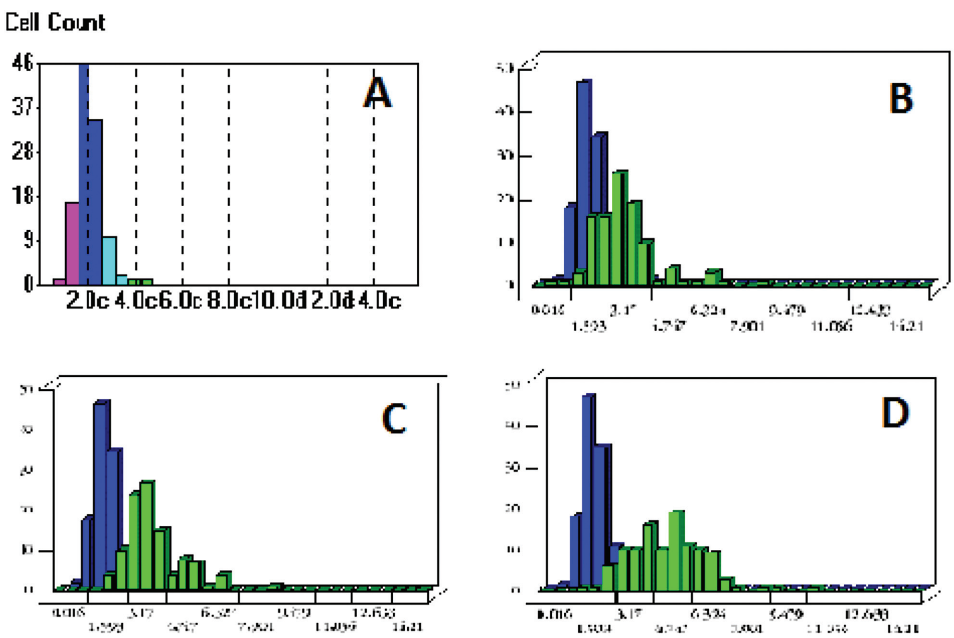
All data are presented as mean±SD. All data with different super script letters (a, b, c, d, e) are significant at P value less than 0.05 using the analysis of variance test.

Table 4 Cell cycle indices of the studied groups

	G1cell %	S cell %	G2 cell %	Aneuploid cell %
Normal colonic mucosa	72.0	22.0	6.0	0.0
Adenomas*				
Low-grade dysplasia	35.8	36.8	18.4	9.0
High-grade dysplasia	19.0	42.5	22.5	16.0
Adenocarcinoma*				
Grade I	7.0	29.7	32.0	31.3
Grade II	1.7	14.3	29.7	54.3
Grade III	1.0	8.4	18.3	72.3

*Significant difference at P value less than 0.05 using the χ^2 test.

Figure 3



Histogram of cell cycle indices: (a) normal colonic mucosa, (b) adenoma with dysplasia, (c) grade I adenocarcinoma, and (d) grade III adenocarcinoma.

In adenoma cases both of proliferating cells % (S+G2) and proportion of bcl-2 positive cells increased with significant correlation in low-grade and high-grade dysplasia. In carcinoma cases both parameters decreased with significant correlation in different histologic grades. As regard aneuploidy cells %, there was a significant inverse correlation with proportion of bcl-2 positive cells in dysplastic adenoma and carcinoma cases with different histologic grades (Table 5).

Discussion

Bcl-2 gene is a 26-kD protein that inhibits apoptosis and may lead to accumulation and propagation of cells having genetic mutation [8]. Apoptosis is a form of programmed cell death that happens in normal tissue development and regeneration. It can be activated by stress signals such as nutrient deprivation, reactive oxygen species and excessive mitogenic signaling associated with tumor initiation [24].

Increased proliferation which occurs due to oncogenic mutations is promoted by alterations in apoptotic pathways that finally lead to tumor growth. Colon apoptosis has a crucial role in intestinal turnover, so that the disruption of balance between apoptosis and proliferation facilitate tumor development and progression [25].

On the other hand, nuclear morphometry and the study of cell cycle by image cytometric analysis are very useful in understanding and predicting tumor development. Dysregulation of the cell cycle is the primary step in carcinogenesis and have an important role in tumor invasion and metastases [21].

This study aimed to evaluate immunohistochemical expression and image analysis of nuclear parameters in premalignant and malignant colon tumors.

In our study, normal mucosa of colon showed bcl-2 positive staining in the lower half of colonic crypts and surface epithelium were negatively stained for bcl-2. These results were also shown by previous studies [26,27]. They demonstrated that bcl-2 is normally expressed in the basal portion of colonic crypts which correspond to the progenitor cells. These cells are known to protect the regenerative compartment from cell death.

In the present study, 85% of the colon dysplasia (low and high grades) is positive for bcl-2 expression, however, its expression showed nonsignificant

Table 5 Correlation between bcl2 expression and nuclear parameters

	Proportion of bcl-2 positively stained cells		
	Low >25%	Moderate 25–50%	High <50%
Nuclear area			
Adenomas			
Low-grade dysplasia	20.4	19.2	16
High-grade dysplasia	21.9	19.7	19.5
Adenocarcinoma*			
Grade I	25.6	24.1	21.3
Grade II	30.6	30	29.1
Grade III	37.6	36.4	31.5
Aneuploid cells			
Adenomas*			
Low-grade dysplasia	11	11	5
High-grade dysplasia	23	14	13
Adenocarcinoma*			
Grade I	37	31	26
Grade II	61	51	51
Grade III	81	69	67
Proliferating cell			
Adenomas*			
Low-grade dysplasia	36	64	65.5
High-grade dysplasia	55	69	69
Adenocarcinoma*			
Grade I	58	60	67
Grade II	38	47	47
Grade III	19	29	32

*Significant difference at *P* value less than 0.05 using the χ^2 test.

correlation with the degree of dysplasia. These results were in accordance with Elsharkawy [22] that showed 61% of cases of colon dysplasia showed bcl-2 immunopositivity with nonsignificant relation with the degree of dysplasia. On the other hand, 65% of colon dysplasia in our study showed high percentage of bcl-2 immunoreactivity (>50%) staining, while 25% had moderate staining (25–50%), and the remaining 15% showed low percentage of bcl-2 staining (<25%) with nonsignificant correlation between percentage of bcl-2 positivity and degree of dysplasia.

The prognostic significance of bcl-2 expression is controversial in colon cancer. While some reports showed a favorable prognostic role of bcl-2 [16,28], others demonstrated that bcl-2 is a poor prognostic marker for malignant tumors including colon cancer [29].

In the current study, bcl-2 positivity was detected in about 53% of CRC with a significant difference between its expression in carcinoma cases and cases of dysplastic adenoma. The percentage of positive cells decrease from low to high grade with inverse correlation with tumor differentiation. These results were in agreement with the study of Patil *et al.* [30] which showed that well-differentiated cases had greater bcl-2 immunoreactivity than poorly differentiated cases. However, a previous study demonstrated that bcl-2 overexpression was associated with high histological grades, resulting in aggressive tumor [29]. Other study did not detect any correlation between bcl-2 expression and histopathological grade [31]. This study showed that the percentage of bcl-2 positivity in grade III is significantly lower than that in grade I and grade II. Similar results were demonstrated by previous studies which showed increased of expression bcl-2 in adenomas than carcinomas indicating its role in the early stages of carcinogenesis [27,32]. Moreover, lack of bcl-2 expression has been associated with invasion, metastases, and recurrence of CRC [28].

The consonance between the antiapoptotic and proapoptotic bcl-2 family proteins is responsible for cell's fate. Studies have been performed to identify bcl-2 inhibitors, and to evaluate the clinical value of using bcl-2 antiapoptotic members as therapeutic targets for many malignant tumors [33].

Various methods can be used for analysis of cell cycle; however, in the present study we used image analysis for nuclear morphometry and cell cycle progression in colon adenoma and carcinoma. Morphometric analysis comprises measuring and counting to obtain accurate data for cell and tissue components that are important for diagnosis and prognosis of several tumors. Quantitative assessment of nuclear morphometry with computerized image analysis was done in many studies and correlated with prognostic factors of various malignancies including colon cancer, breast cancer, and renal cell carcinoma [34–36].

In the present study, all nuclear parameters showed nonsignificant increase in values with increasing dysplastic changes in cases of adenomas, while in colon carcinoma only nuclear area showed significant increase with increasing histologic grade ($P<0.01$). On the other hand, nuclear area showed statistically significant difference ($P<0.01$) between adenoma with high-grade dysplasia and grade I colon adenocarcinoma. This is in accordance with Yassen *et al.* [37] who suggested that nuclear area has a considerable role in prediction of carcinoma. They added that nuclear morphometry has also an important role in screening of patients with colon cancer who are at risk of metastases or recurrence after curative surgery.

Nuclear morphometric studies were verified in previous studies of various malignancies [38–40]. Mendaçolli *et al.* [41] detected significant changes in nuclear morphometric and chromatin texture pattern between basal cell carcinoma and normal basal epithelium. Other studies showed a significant difference in nuclear morphometric parameters including perimeter, circularity, and major and minor axes ratio between hepatocellular carcinoma and adjacent tissue hepatocytes with high sensitivity and specificity for discrimination between neoplastic and nonneoplastic hepatocytes [42,43]. They determined that the incorporation of technological digital histological analysis for nuclear morphometry can help the pathologists in quantifying parameters that may not be detected by a subjective analysis.

The cell cycle is divided into four phases; the length of these cell cycle phases is an important characteristic of cell life [19]. The cell progression through these phases is controlled by endogenous and exogenous factors. On the other hand, the relationship between cell division and cell death represents a link between cell cycles and cell death programs that is present in all cells. Cancer is characterized by aberrant regulation, leading to uncontrolled proliferation and replicative immorality [44].

In our study of cell cycle by image cytometry, the examined cells in G1 phase showed significant decrease ($P<0.01$) from control cases, low-grade dysplasia, high-grade dysplasia to cases of carcinomas with increasing grades. In adenomas there is a significant increase in the percentage of euploid proliferating cells than in normal colonic mucosa. However, in carcinomas, the percentage of euploid proliferating cells decreases with advanced tumor grade, this was shown as large percent of cell population shift to the aneuploid category outside the normal cell cycle. Both S phase and G2 values increase significantly in carcinomas.

Previous flow cytometric study of DNA [45] in CRC suggested that DNA content of tumor cells is important for prognosis. The flow cytometric results showed that high S-phase values indicate that a large proportion of cells are proliferating, resulting in rapid growth of tumor. The study also demonstrated that DNA content, S-phase fraction, and tumor ploidy determination reveal important information about the biological behavior of colon cancer.

In accordance with our study, Elsharkawy [22] observed that DNA aneuploidy was more frequently detected in CRC than adenomas with statistically significant difference. They also demonstrated that there was a significant correlation between aneuploidy and degree of dysplasia in adenomas and increasing histological grades of carcinoma, suggesting the role of aneuploidy in predicting the malignant potential of adenoma.

Other studies showed that S-phase values also have a prognostic role in tumors of lymphatic system and breast and may be useful in predicting response to treatment in head and neck malignancies [46,47].

Cell death initiated by cell cycle checkpoint proceeds through apoptosis. Apoptosis is the form of cell death during development. It is clear that, the link between cell death and proliferation, is between apoptosis and the cell cycle [48]. In the present study, the percentage of proliferating cells (S+G2 phases) increased and showed significant correlation with the proportion of bcl-2 positive cells in cases of colon adenoma (low-grade and high-grade dysplasia). On the contrary both parameters decrease in cases of colon carcinoma with significant correlation between the percentage of aneuploid cells and proportion of bcl-2 positive cells. These results point toward the role of altered expression of bcl-2 that occurs in the sequence of molecular events of cell cycle leading to colon carcinoma. Therefore, many preclinical studies supported the clinical potential of bcl-2 inhibitors as single factor or in combination with other standard therapies to improve treatment responses and patient survival [33,49–52].

Finally, early diagnosis and prediction of patients at high risk for developing CRC is important. Therefore, data from computerized morphometry and cell cycle analysis together with immunohistochemistry have a considerable role in detecting the ability of high dysplastic colon cells to be turned into malignant cells.

Conclusion

Bcl-2 protein immunoreactivity play an important role in early event of colorectal carcinogenesis and its inhibitor may have a potential use in management of colon cancer. The acceptable reliability of immunohistochemical, nuclear area, and cell cycle analysis may serve as diagnostic and prognostic indicators in benign and malignant colorectal lesions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. Cancer J Clin 2021; 71:7–33.

2 Kunac N, Filipovic N, Kostic S, Vukojevic K. The expression pattern of Bcl-2 and Bax in the tumor and stromal cells in colorectal carcinoma. Medicina 2022; 58:1135–1145.

3 Lalier L, Vallette F, Manon S. Bcl-2 family members, and the mitochondrial import machineries: the roads to death. Biomolecules 2022; 12:162–181.

4 Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, *et al.* Cancer treatment and survivorship statistics 2016. CA Cancer J Clin 2016; 66:271–289.

5 Ali I, Lone MN, Al-Othman ZA, Al-Warthan A, Sanagi MM. Heterocyclic scaffolds: centrality in anticancer drug development. Curr Drug Targets 2015; 16:711–734.

6 Tong G, Zhang G, Liu J, Zheng Z, Chen Y, Niu P, *et al.* Cutoff of 25% for Ki67 expression is a good classification tool for prognosis in colorectal cancer in the AJCC8 stratification. Oncol Rep 2020; 43:1187–1198.

7 Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol 2014; 15:49–63.

8 Bhardwaj M, Mardi K, Kaushal V, Sharma M, Rao M. Bcl-2 expression in colorectal carcinoma and its correlation with clinicopathological parameters. Clin Cancer Investig J 2020; 9:182–183.

9 Luna-Vargas MP, Chipuk JE. The deadly landscape of pro-apoptotic BCL-2 proteins in the outer mitochondrial membrane. FEBS J 2016; 283: 2676–2689.

10 Gabellini C, Trisciuoglio D, Del Bufalo D. Non-canonical roles of Bcl-2 and Bcl-xL proteins: relevance of BH4 domain. Carcinogenesis 2017; 38: 579–587.

11 Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ 2018; 25:65–80.

12 Adams JM, Cory S. The BCL-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death Differ 2018; 25:27–36.

13 Tsamandas AC, Kardamakis D, Petsas T, Zolota V, Vassiliou V, Matatsoris T, *et al.* Bcl-2, bax and p53 expression in rectal adenocarcinoma. Correlation with classic pathologic prognostic factors and patients' outcome. In Vivo 2007; 21:113–118.

14 Torsello A, Garufi C, Cosimelli M, Diodoro MG, Zeuli M, Vanni B, *et al.* Colorectal disease management team, Regina Elena Cancer Institute, Rome, Italy. P53 and bcl-2 in colorectal cancer arising in patients under 40 years of age: distribution and prognostic relevance. Eur J Cancer 2008; 44:1217–1222.

15 Fucini C, Messerini L, Saieva C, Orzalesi L, Carroni V, Bartolini N. Apoptotic proteins as prognostic markers and indicators of radiochemosensitivity in stage II/III rectal cancers. Colorectal Dis 2012; 14:e64–e71.

16 Balzi M, Ringressi MN, Faraoni P, Booth C, Taddei A, Boni L, *et al.* B-cell lymphoma 2 and β-catenin expression in colorectal cancer and their prognostic role following surgery. Mol Med Rep 2015; 12:553–560.

17 Melincovici CS, Mihu CM, Mărginean M, Boşca AB, Coneac A, Moldovan I, *et al.* The prognostic significance of p53, Bax, Bcl-2 and cyclin E protein overexpression in colon cancer – an immunohistochemical study using the tissue microarray technique. Rom J Morphol Embryol 2016; 57:81–89.

18 Kashyap A, Jain M, Shukla S, Andley M. Role of nuclear morphometry in breast cancer and its correlation with cytomorphological grading of breast cancer: a study of 64 cases. J Cytol 2018; 35:41–46.

19 Ligasová A, Frydrych I, Koberna K. Basic methods of cell cycle analysis. Int J Mol Sci 2023; 24:3674.

20 Malumbres M, Barbacid M. Cell cycle CDKs and cancer: a changing paradigm. Nat Rev Cancer 2009; 9:153–166.

21 Krabbe LM, Margulis V, Lotan Y. Prognostic role of cell cycle and proliferative markers in clear cell renal cell carcinoma. Urol Clin N Am 2015; 43:105–118.

22 Elsharkawy SL. Bcl-2 immunoreactivity and DNA ploidy in colorectal neoplasia: correlation with histologic grade. Egypt J Natl Res Centre 2006; 5:10–6.

23 Sharma AK, Sharma A. Chromosome Techniques: Theory and Practice, Third Edition, 1980.

24 Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol 2019; 20:175–193.

25 Ramesh P, Medema JP. BCL-2 family deregulation in colorectal cancer: potential for BH3 mimetics in therapy. Apoptosis 2020; 25:305–320.

26 Poincloux L, Durando X, Seitz JF, Thivat E, Bardou VJ, Giovannini MH, *et al.* Loss of Bcl-2 expression in colon cancer: a prognostic factor for recurrence in stage II colon cancer. Surg Oncol 2009; 18:357–365.

27 Kumar AKKT, Saldanha P. Bcl-2 analysis in colorectal carcinoma – an immunohistochemical study. Int J Innov Res Med Sci 2017; 2:2455–8737.

28 Huang Q, Li S, Cheng P, Deng M, He X, Wang Z, *et al.* High expression of anti-apoptotic protein Bcl-2 is a good prognostic factor in colorectal cancer: result of a metaanalysis. World J Gastroenterol 2017; 23:5018–5033.

29 Maather BHA. Immunohistochemical study of CEA and BCL2 expression in colorectal adenocarcinoma and its correlation with some pathological parameters. Med J Babylon 2011; 8:243–252.

30 Patil ST, Wilfred CD, Shetty PB. Histopathological evaluation and analysis of immunohistochemical expression of Bcl-2 oncoprotein in colorectal carcinoma. Iran J Pathol 2019; 14:317–321.

31 Qasim B, Ali H, Hussein A. Immunohistochemical expression of p53 and Bcl-2 in colorectal adenomas and carcinomas using automated cellular imaging system. Iran J Pathol 2012; 7:215–223.

32 Petrişor O, Giuşcă SE, Sajin MA, Dobrescu G, Căruntu ID. Ki-67, p53 and bcl-2 analysis in colonic versus rectal adenocarcinoma. Rom J Morphol Embryol 2008; 49:163–171.

33 D'Aguanno S, Del Bufalo D. Inhibition of anti-apoptotic Bcl-2 proteins in preclinical and clinical studies: current overview in cancer. Cells 2020; 9:1287–1312.

34 Ikeguchi M, Sakatani T, Endo K, Makino M, Kaibara N. Computerized nuclear morphometry is a useful technique for evaluating the high metastatic potential of colorectal adenocarcinoma. Cancer 2000; 86:1944–1951.

35 Yamamoto Y, Saito A, Tateishi A, Shimojo H, Kanno H, Tsuchiya S, *et al.* Quantitative diagnosis of breast tumors by morphometric classification of microenvironmental myoepithelial cells using a machine learning approach. Sci Rep 2017; 7:46732.

36 Agrawal S, Jain N. Nuclear morphometry is a superior prognostic predictor in comparison to histological grading in renal cell carcinoma: a retrospective clinico-pathological study. Indian J Pathol Oncol 2022; 9:208–215.

37 Yassen NN, Abouelfadl DM, Gamal elDin AA. Morphometric analysis and immunohistochemical expression of cytochrome C oxidase in colonic adenomas and adenocarcinomas. J Arab Soc Med Res 2018; 13:119–128.

38 Mijovic Z, Kostov M, Mihailovic D, Zivkovic N, Stojanovic M, Zdravkovic M. Correlation of nuclear morphometry of primary melanoma of the skin with clinicopathological parameters and expression of tumor suppressor proteins (p53 and p16(INK4a)) and bcl-2 oncoprotein. J BUON 2013; 18:471–477.

39 Nishihira M, Nakazato Y, Tsuchida S, Kuroda H, Imai Y, Chida M. Nuclear morphometry of lung squamous cell carcinomas in cytologic study. Ann Thorac Cardiovasc Surg 2018; 24:303–307.

40 Nunes TWN, Filippi-Chiela EC, Callegari-Jacques SM, da Silva VD, Sansonowicz T, Lenz G, *et al.* Nuclear morphometric analysis in tissue as an objective tool with potential use to improve melanoma staging. Melanoma Res 2019; 29:474–482.

41 Mendaçolli PJ, Brianezi G, Schmitt JV, Marques MEA, Miot HA. Nuclear morphometry and chromatin textural characteristics of basal cell carcinoma. An Bras Dermatol 2015; 90:874–878.

42 Hassan S, Mansy SS, Tabak SA, Abdelfattah AS, Ahmed MA, Hamam O, *et al.* Immunohistochemical and electron microscopic morphometric image analysis of hepatocellular carcinoma in association of HCV infection. Ultrastruct Pathol 2018; 42:97–107.

43 Dos Santos JB, Starosta RT, Pilar EFS, Kunz JD, Tomedi J, Cerski CTS, *et al.* Nuclear morphometry and chromatin texture changes in hepatocellular carcinoma samples may predict outcomes of liver transplanted patients. BMC Gastroenterol 2022; 22:189.

44 Loftus LV, Amend SR, Pienta KJ. Interplay between cell death and cell proliferation reveals new strategies for cancer therapy. Int J Mol Sci 2022; 23:4723–4746.

45 Hixon C, Furlong J, Silbergleit A. Flow cytometry in colon cancer: does flow cytometric cell cycle analysis help predict for short-term recurrence in patients with colorectal carcinoma? J Natl Med Assoc 1995; 87:803–806.

46 Pucci B, Kasten M, Giordano A. Cell cycle and apoptosis. Neoplasia 2000; 2:291–299.

47 Zheng K, He Z, Kitazato K, Wang Y. Selective autophagy regulates cell cycle in cancer therapy. Theranostics 2019; 9:104–125.

48 Voss AK, Strasser A. The essentials of developmental apoptosis. F1000Res 2020; 9:F1000.

49 Garner TP, Lopez A, Reyna DE, Spitz AZ, Gavathiotis E. Progress in targeting the BCL-2 family of proteins. Curr Opin Chem Biol 2017; 39:133–142.

50 Amato J, Pagano A, Capasso D, Di Gaetano S, Giustiniano M, Novellino E, *et al.* Targeting the BCL2 gene promoter G-quadruplex with a new class of furopyridazinone-based molecules. Chem Med Chem 2018; 13: 406–410.

51 Gunaratnam M, Collie GW, Reszka AP, Todd AK, Parkinson GN, Neidle SA. naphthalene diimide G-quadruplex ligand inhibits cell growth and down-regulates BCL-2 expression in an imatinib-resistant gastrointestinal cancer cell line. Bioorg Med Chem 2018; 26:2958–2964.

52 Warren CFA, Wong-Brown MW, Bowden NA. BCL-2 family isoforms in apoptosis and cancer. Cell Death Dis 2019; 10:177–189.