Identification of KI67 & BCL-2 Proteins Among Sudanese Patients Suffering From Colorectal Cancer

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Abstract: The growth of tumors is highly variable and this probably reflects even its clinical course. where found that the expression of ki67 in this study give positive result in only 16 persons, negative 22 person and week in 12 persons. However, as reported so far the Dukes stage show ki67 positive in dukes stage C positive 6, negative 9 and week 4, in Dukes B positive 5, negative 9 and week 7. In Dukes a positive 2and negative 1, Dukes D positive 3, negative 3 and week 1. Bcl-2 expression appears to be strictly associated, the bcl-2 give positive result in 26 patients and negative in 17 patients also give week result in 7 patient. . And we see increase of number of colorectal cancer against the age where in younger age the danger is low 5 people between age 25 – 35 and high between age 50 – up about 32 person.

Keywords: about seven key words separated by commas.

1. Literature Review

Colorectal cancer is the third most common type of cancer and the fourth most frequent cause of death due to cancer worldwide[1]. Worldwide mortality attributable to colorectal cancer is approximately half that of the incidence. Almost one million new cases occurred annually[2]. Colorectal cancer survival is highly dependent upon the stage of disease at diagnosis, and typically ranges from a 90% 5-year survival rate for cancers detected at the localized stage; 70% for regional; to 10% for people diagnosed for distant metastatic cancer[3] .In general the earlier the stage at diagnosis, the higher the chance of survival, but unfortunately the disease is very often diagnosed only at an advanced stage, and prognosis is accordingly poor Various screening methods for detection of tumors are available but their diagnostic and prognostic values are limited regarding sensitivity (fecal occult blood test) or regarding costs, risks, and inconvenience (colonoscopy)[9]. Causes of colon cancer often are environmental in sporadic cases (80%) and sometimes genetic (20%). Since malignant cells have a changed genetic makeup, this means that in 80% of cases, the environment spontaneously induces change, whereas those born with a genetic predisposition are either destined to get the cancer or less environmental exposure can induce the cancer. Exposure to agents in the environment that may induce mutation is the process of carcinogenesis and is caused by agents known as carcinogens (cancer-causing agents) [4]. Specific carcinogens have been difficult to identify; however, dietary factors seem to be involved. Colon cancer is more common in industrialized nations. Diets high in fat, red meat, total calories, and alcohol seem to predispose people to the disease[10]. Two-thirds of all cases occur after age 50 and the average age for those who develop the disease is 62. There also is a slight increased risk for colon cancer in the individual who smokes[11]. Patients who suffer from inflammatory diseases of the colon known as ulcerative colitis and Crohn's colitis are also at increased risk[5].

Neuploid tumors are commonly associated with poor prognosis, although a lack of correlation between a DNA ploidy pattern and patient outcome has also been reported Furthermore[12], the clinical usefulness of cell proliferative activity, evaluated either as percentage of cells in S-phase by flow cytometry or immuno-histochemically, is debated. As for other human malignancies, the development of colorectal adenocarcinoma is associated with a series of inherited and/or acquired gene abnormalities that dysregulate cell growth. One of the oncogenes implicated in this process is ***Bcl-2***, the products of which play a crucial role in apoptosis, cell proliferation and tumor progression. Bcl-2 inhibits apoptosis both in normal and transformed cells[13]. In colorectal cancer, Bcl-2 protein expression has been related to tumorigenesis[6]. There are a few, and often contradictory, studies which have investigated the correlation between Bcl-2 and patient outcome[8]. Indeed, in colorectal carcinomas, IHC expression of this anti-apoptotic protein may be associated with increased survival. Ki67 are weakly expressed in normal tissues but abundant in malignant tumor tissue[14], facilitating tumor metastasis, which is highly sensitive markers for endothelial cell differentiation and have also been studied as markers for tumor invasiveness[7].Another modification of the original Dukes classification was made in 1935 by Gabriel, Dukes and Bussey.[9] This subdivided stage C. This staging system was noted to be prognostically relevant to rectal and colonic adenocarcinoma.[10] Stage D was added by Turnbull to denote the presence of liver and other distant metastases [11].

- Stage A: Limited to muscularis propria; nodes not involved
- Stage B: Extending beyond muscularis propria; nodes not involved
- Stage C: Nodes involved but highest (apical) node spared
- Stage D: Distant metastatic spread

In this study, we analyzed the association of Bcl-2 and Ki67 expressions in colorectal cancer tissues, histo-differentiation, as

well as location, and evaluated the clinical significance of these markers in the prediction of colorectal cancer progression.

2. Materials & Method

2.1 Patients characteristics

A group of 50 patients surgically treated for colorectal adenocarcinoma at the Ibn sena abdominal hospital between 2013 and 2016 has been included in our study. Here were 28males and 22 females with a median age of 47.5 (range: 28–67) years of these study.11 patients underwent radical surgery intended as a resection with clear pathological margins and regional lymphadenectomy. Nerve-sparing and complete mesorectal excision was performed. The same surgical staff carried out all the operations, and no operative mortality was registered. Multiple samples (one in the center and at least 4 at the peripheral edges of the lesion) of fresh tissues from both tumor and pathologically healthy. Tumor tissues were pathologically staged according to the modified Dukes' classification as follows: 3 stage A (6%), 21 B (42%), 19 C (38%) and 7 D (14%).Tumors was categorized according to the WHO classification, as well differentiated (G1) 6 cases (12%), moderate (G2) 37 cases (74%) and poor (G3) 7 cases (14%).

2.2 Tissue Microarray block

Microarray is a technique for organizing minute amounts of biological samples on a solid support.[16] Tissue microarrays are composite paraffin blocks constructed by extracting cylindrical tissue core "biopsies" from different paraffin donor blocks and re-embedding these into a single recipient (microarray) block at defined array coordinates.[17, 18] Firstly, the donor blocks (invariably stored paraffin blocks) are retrieved and sectioned to produce standard microscopic slides that are stained with hematoxylin and eosin. An experienced pathologist examines the slides to mark the area of interest, which is commonly an area of cancer, after which the samples can be arrayed, in this research we used microarray block 8 – 4 (32 samples in each block), which will used tonsil as positive control for both ki67 and BCL-2 immunohistochmistry technique. If tissue arrays are constructed from tumor tissues, the targeting of a specific area representative for the specimen is crucial for the quality of the array. Hematoxylin/eosin-stained full sections from the donor blocks well obtained to assess morphology and to identify an area that represents the specimen. This can vary greatly among tissue types. For example, in colorectal cancer it is important that the target areas are small and well defined because stromal areas between the glandular structures of the tumor can be large and a core biopsy can easily miss the tumor-cell-rich regions. The area of the donor block to be cored for TMA well be selected by a pathologist.

2.3 Immunohistochemistry

Immunohistochemical study was performed in all tissue samples, using ultrasensitive kit and diaminobenzidine, as recommended by the manufacturers. Briefly, sections (4 μm, thickness) were deparaffinized with xylene, rehydrated through graded alcohol, and rinsed in phosphate-buffered saline (PBS). Antigen retrieval was performed by placing the slides in boiling citric acid buffer at pH 6.0 for 5 min. Sections were then sequentially incubated in 3% H2O2 in methanol for 20 min at room temperature to quench endogenous peroxidase activity, non immune serum albumin to block the nonspecific binding, primary antibodies and anti-Ki67 or anti BCL-2 at 4°C overnight. For the subsequent reaction, the tissue sections were treated with biotinylated secondary antibody, followed by further incubation with streptavidin–horseradish peroxidase complex. Intervening PBS washing was necessary between each two steps. Immunoreaction was visualized with diluted DAB. Finally, sections were rinsed with distilled water, counterstained with Mayer's hematoxylin, and mounted with histomount. The negative controls were set by superseding the primary antibodies. The positive controls were the Tonsil cancer with both ki67 and BCL-2.

2.3.1 Evaluation of immunohistochemistry

Ki67 staining is mainly located in the nuclear of colorectal proliferative cells. Only cells with a distinct nuclear Ki-67 staining were considered as positive, and the percentage of immunoreactive nuclei was calculated by counting in a series of randomly selected 10 microscopic fields (corresponding to a total of at least 100 tumor cells) under high-power magnification (×400).for statistical analyses, the staining results were categorized into three groups (week, moderate, high). According to the percentage of ki 67 positive as follows: low ki67 0% - 10%, moderate more than 10% and up to 25%, high ki67, 25% and more. Immunoreactivity for bcl-2 was evaluated according to the percentage of tumor cells with positive cytoplasmic staining. A cut-off <5% of positive tumor cells was used to define negative cases. Strong positive staining was seen in infiltrating lymphocytes within the tumor stroma. Randomly selected 10 microscopic fields (corresponding to a total of at least 100 tumor cells) under high-power magnification (×400).

2.4Statistical analysis

All data were analyzed by statistics software (SPSS 13.0 for Windows; SPSS, Inc.). Chi-square test and Fisher exact test were used to compare the levels of ki67 and bcl-2 expressions with both different groups and various clinicopathologic parameters. Bivariate correlations between two independent variables were analyzed by calculating the Spearman's correlation coefficients. Survival analysis was performed using the Kaplan–Meier method and compared by the log-rank test. Prognostic relevance was evaluated by multivariate Cox regression analysis. $P < 0.05$ was considered as significant. Full Dukes' classification

3.0 Results

A total of 50 samples of known colorectal cancer . which showing as dukes stage **A** (6%) , stage **B** (42%), stage **C** (38%) , stage **D** (14%). As Show in table **1**. Statistical analysis of the results revealed no statistically significant correlations of Bcl-2 and Ki-67 expression with gender or age of patients show in

table **5**. Histological type or distant metastasis. However, we observed a correlation of PCNA (***p***=0.032).For the rectal adenocarcinoma (Table 2): from the well differentiated form (12%) to the poor differentiated form (14%); the moderately differentiated form had an (74%).

Ki-67 protein is present during all active phases of the cell cycle, in this study we divide the result in to 3 types for each marker (positive, week and negative),in ki67 fallowed as positive ki67 **(32%)**, negative ki67 **(44%)** and week ki67 **(24%)** as show in table **3**.Bcl-2 was evaluated according to the percentage of tumor cells with positive cytoplasmic staining of bcl-2 and given results fallow as positive 26 (52%), negative 17 (34%) and week only 7 (14%), which as show in table **4.**

The correlation between ki67 and bcl-2 and age fallowed as age from 25 – 35 (5 persons 10% of total number), 35 – 50 (13persons 26% of total number), 50 – up (32 persons 64% of total number) as show in Table **5**. There were 21 women (42%) and 29 men (58%) in the group as show in Table **6**, aged 27 - 82 years, in the study group.The positive IHC for ki67 was show in figure (1), and the negative as show in figure (2), the positive BCL-2 as show in figure (3), negative as show in figure (4).

Table 1 showing the frequency of colorectal cancer depending on the types of dukes:

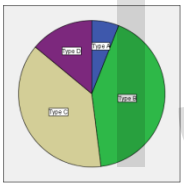

Figure 1

SAMPLES (adenocarcinoma)	FREQUENCY	PERCENT
Well Differentiated	*6*	*12.0*
Moderate Differentiated	*37*	*74.0*
Poor Differentiated	*7*	*14.0*

Table 2 showing the frequency of colorectal cancer according to the degree of differentiation of colonic adenocarcinoma

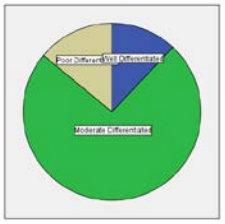

Figure 2

Ki67	Dukes C	Dukes B	Dukes A	Dukes D	Total	PERCENT
Positive	6	5	2	3	16	32
Negative	9	9	1	3	22	44
Week	4	7	0	1	12	24

SAMPLES (dukes types)	FREQUENCY	PERCENT
Type A	*3*	*6.0*
Type B	*21*	*42.0*
Type C	*19*	*38.0*
Type D	*7*	*14.0*
Total	50	100

Table 3 showing the frequency of ki67 against the type of dukes:

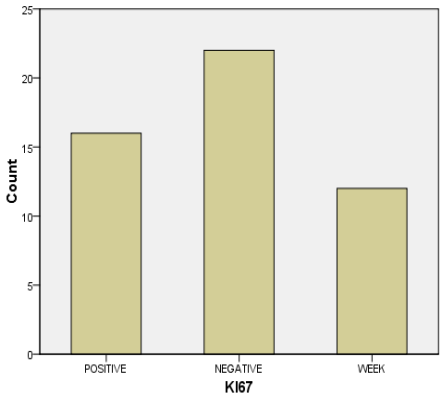

Figure 3

Bcl-2	Dukes				Total	Percent
	C	B	A	D		
Positive	10	12	1	3	**26**	**52**
Negative	6	6	1	4	**17**	**34**
Week	3	3	1	0	**7**	**14**
Total	19	21	3	7	**50**	**100**

T able 4 showing the frequency of bcl-2 result against the types of dukes:

Age	Ki67			Total	Bcl-2			Total
	Positive	Negative	Week		Positive	Negative	Week	
25 - 35	1	3	1	**5**	2	3	0	**5**
35 - 50	4	6	3	**13**	8	3	2	**13**
50 - up	11	13	8	**32**	16	11	5	**32**
Total	16	22	12	**50**	26	17	7	**50**

Table 5 show the correlation between ki67, bcl-2 and age:

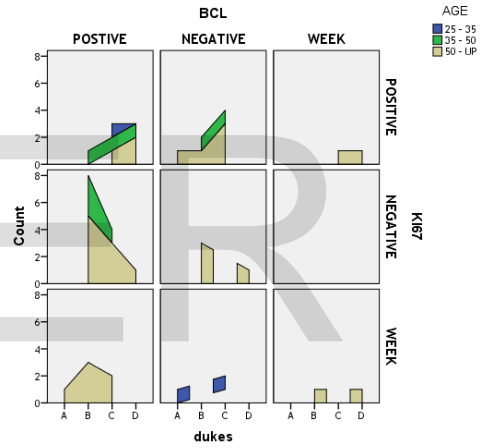

Figure 5

Table 6 show the correlation between sex and dukes:

Sex	*Dukes*				*Total*	Percent
	C	B	A	D		
Male	7	17	1	4	***29***	**58**
Female	12	4	2	3	***21***	**42**
Total	19	21	3	7	***50***	**100**

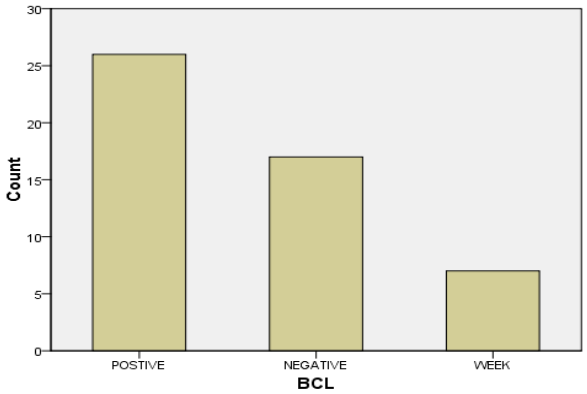

Figure 4

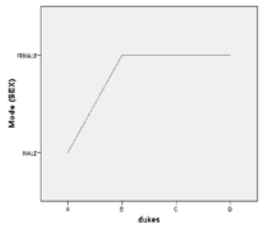

Figure 6

4.0 DISCUSSION

Our finding is in agreement with study done by Azza Hegazy, Sahar A. Daoud Academic Journal of Cancer Research 7 (3): 168-

172, 2014 for bcl-2, However in agreement different studies there was a highly significant relation between Ki67 marker, the tumor grade and stage. This ascertains is in consensus with the literature data, underlying the concept of colorectal cancers as a whole [18, 19].where found that the expression of ki67 in this study give positive result in only 16 persons, negative 22 person and week in 12 persons. However, as reported so far the Dukes stage show ki67 positive in dukes stage C positive 6, negative 9 and week 4, in Dukes B positive 5, negative 9 and week 7. In Dukes a positive 2and negative 1, Dukes D positive 3, negative 3 and week 1. Bcl-2 expression appears to be strictly associated. And we see increase of number of colorectal cancer against the age where in younger age the danger is low 5 person between age 25 – 35 and high between age 50 – up about 32 person. And increase the number of patients in male more than female. Recent studies established the fact that an increased expression of Ki-67 indicates a better survival in rectal and recto-sigmoid cancer[20], ki67 has a prognostic and/or predictive value in different tumor types (16). Recent studies, established the fact that an increased expression of ki67 indicates a better survival in rectal and recto sigmoid cancer as these tumors have better response to radiotherapy. However in agreement different studies there was a highly significant relation between ki67 markers, the tumor grade and stage.Bcl-2 is a gene involved in the cell cycle regulation by inhibiting apoptosis, anti apoptotic onco-protein in some cell systems under physiological and neoplastic condition. The role of bcl-2 in colorectal tumor of carcinogenesis. A decrease in the levels of bcl-2 can lead to cell death by apoptosis while it's over expression protects against programmed cell death. A significant association was found between bcl-2 expression in our studied cases and tumor grade and stage, show more expression than ki67.however, the bcl-2 give positive result in 26 patients and negative in 17 patients also give week result in 7 patient,

On the basis of partial correlation coefficients analysis calculated in the multiple correlation, it was possible to evaluate the degree of association of every marker with the adenocarcinoma location (colonic vs. rectal). Consequently, we obtained a strong reverse correlation between ki67 and location (p-0000, 95%CL) .the higher value of bcl-2 and ki67 being associated with colonic adenocarcinoma. The statistic analysis also focused on the correlation between the investigated markers as prediction factors prognosis. Differentiation degree of tumor (pathologic subtypes of adenocarcinoma) and colonic/rectal location.

Our study confirms, from the statistical point of view, the predictive value of bcl-2 marker, followed by proliferation index ki67.the result obtained for ki67 marker did not permit its validation as prognosis factor.

5.0 Conclusion and recommendation

The evaluation of Ki-67 and bcl-2 yields refined information on colorectal tumor biology as we noticed statistically significant relations with tumor grade and stage. This could be integrated with the clinical and biologic tumoral framework for good assessment of the studied cases. We live in third world countries so the education is very low and cultural concept may influence main causes for late diagnosis of disease. So we must make Voluntary campaigns for screening to learn more about the cancer and other disease. We need more diagnostic test and more research to boost the knowledge about cancer

6.0 Appendix

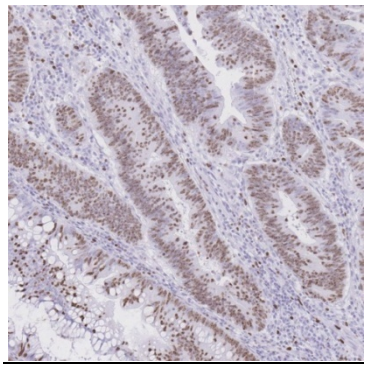

Figure (1)

High magnification of colon cancer (adenocarcinoma) showing expression of the proliferation marker Ki-67 (MKI67) in tumor cells. This particular tumor shows a high grade of proliferation with positive nuclear staining in virtually all tumor cells

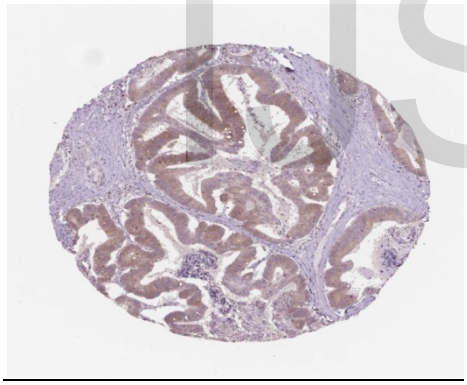

Figure (2)

High magnification of colon cancer (adenocarcinoma) showing expression of the proliferation marker BCL-2 in tumor cells

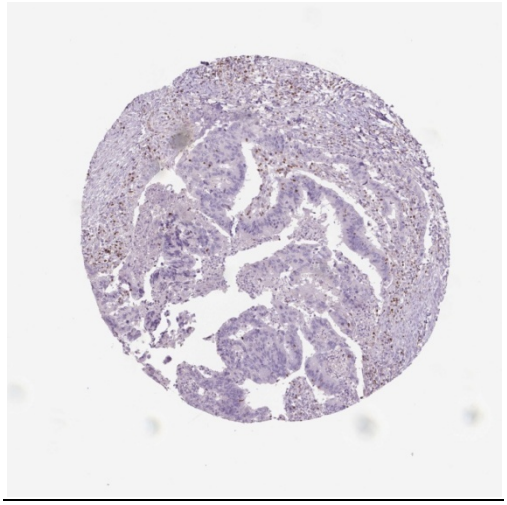

Figure (3)

Low magnification of colon cancer (adenocarcinoma) showing no expression of the proliferation marker of BCL-2 in tumor cells

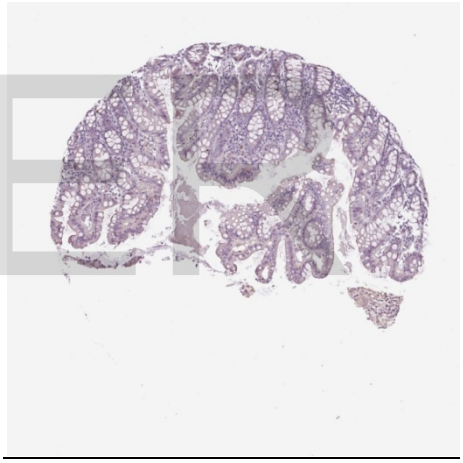

Figure (4)

High magnification of colon cancer (adenocarcinoma) showing no expression of the proliferation marker Ki-67 (MKI67) in tumor cells

7.0 References

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