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Expression of Survivin in Colorectal Tumors

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Abstract:

This study aimed to detect the expression of survivin in colorectal tumors using immunohistochemical method. Fifty formalin fixed paraffin blocks (FFPB) previously diagnosed as colorectal tumors (40 of them were malignant colorectal tumors and 10 were benign colorectal tumors) were used in this study. Blocks were cut and stained by immunohistochemical method for detection of survivin. The age of patients ranged between 20 and 80 years with mean age 47 years. immunohistochemical analysis showed that survivin expression was positive in 8(16%) samples and negative in 42(84%) samples. 8(16%) positive samples were malignant , in contrast no expression was observed in benign tumor and there was no statistical association between survivin expression and histological diagnosis of the tumors (P=0.123). The relation between histological differentiation and survivin expression revealed that 1/9sample was well differentiated tumor, 7/24 samples were moderate differentiated tumor and 0/7

sample was poor differentiated tumor with no significant statistical association (P=0.178). The relation between metastasis and survivin expression revealed 23/40 samples were negative metastasis and 17/40 samples were positive metastasis with no significant statistical association (P=0.201).

Conclusion: The study concluded that the survivin expression in colorectal tumors was not correlated with histological diagnosis, tumor differentiation and tumor metastasis.

Key words: Colorectal Cancer, Survivin

INTRODUCTION:

Colorectal cancer (CRC) is one of the three most common cancers in the world and is a major contributor to cancer-related death [(1)].

Reports from Middle-Eastern countries showed a higher prevalence of CRC in patients undergoing colonoscopy than in the West. CRC was detected in 2.1% of patients who underwent colonoscopy in the United Kingdom and 9–11% in Morocco and Sudan [(2)(3) (4)].

CRC was divided into sporadic (70-80%) and familial cases. Approximately 5 - 10% of all cancers fall into the familial category [(5)]. The vast majority of CRC developed from benign precursor lesions through a series of genetic and epigenetic changes [(6)]. According to the WHO classification, nearly 85% of CRCs were usually adenocarcinomas, and 10 to 15% were classified as mucinous adenocarcinomas. [(7)].

Diet and lifestyle factors are implicated risk factors for the disease. Fruit and vegetable-deficient diet, calorie-dense foods, physical inactivity, obesity and smoking increase the risk for developing colorectal cancer [(8)].

Diagnosis of colorectal cancer is via tumor biopsy typically done during sigmoidoscopy or colonoscopy [(9)]. Treatment advances such as standardized technique of total

mesorectal excision; preoperative radiotherapy and adjuvant chemotherapy have reduced the earlier high local recurrence rates and have improved the survival in patients [10]. However, a great number of patients who underwent apparently curative surgery develop local recurrences or distant metastases leading to shorter survival [11].

Survivin was originally identified by structural homology to inhibitors of apoptosis proteins in human B-cell lymphomas. It is a multifunctional protein implicated in the control of cell proliferation, inhibition of apoptosis and the promotion of angiogenesis [12] [13]. Survivin, as an inhibitor of apoptosis directly inhibits caspase-3 and -7 activities and regulates the cell cycle in the G2/M phase [14].

Survivin is expressed during embryonic and fetal development, is down regulated in normal adult tissues and is over expressed in a variety of human cancers [15][16].

The over expression of survivin in cancer may obliterate this apoptotic checkpoint and allow aberrant progression of transformed cells through mitosis. Survivin expression has been associated with increased aggressiveness and decreased patient survival in a number of different malignancies [17].

During colorectal tumorigensis, surviving protein expression is significantly and progressively increased during the transition from low dysplasia adenoma to high dysplasia carcinoma [13].

MATERIALS AND METHODS:

Sample collection:

Paraffin embedded tissue blocks previously diagnosed as colorectal tumors were collected for this study from different hospitals in Khartoum state.

Slide preparation and staining:

Sections (3µm) from formalin-fixed, paraffin-embedded blocks was cut and mounted onto salinized slides (Fisher brand). Following deparaffinization in xylene, slides were rehydrated through a graded series of ethanol (100%, 90%, 70%, and 50%) and were placed in running water. Samples were steamed for antigen retrieval using PT link. Briefly, slides were placed in slide tank containing enough sodium citrate buffer (pH 9.0) to cover the sections, then were boiled at high temperature for 20 minutes then were allow sections to cool at RT. endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 minutes, then slides were incubated with 100µl of primary anti survivin to each slide for 20 min at room temperature in a moisture chamber, and then were rinsed in phosphate buffer saline. After washing with PBS for 3 min, binding of antibodies were detected by incubating for 20 minutes with dextran labeled polymer (Thermo kit). Finally, the sections were washed in three changes of PBS, followed by adding 3,3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. then counterstained in Mayer's haematoxylin for 1 minute, then washed in water and blued in running tap water for 10 minutes, then dehydrated through ascending of ethanol (70%, 90% and 100%) rinse for each, then cleared in 2 change of xylene 2 minutes for each, and mounted in DPX mounting media.

Positive survivin staining was identified in form of brown nuclear or cytoplasmic staining.

Result Interpretation:

Results obtained were detected by researcher and confirmed by experienced histopathologist. Negative and positive controls were used for evaluation of the test sections.

Data analysis:

The data were analyzed using version 11.5 SPSS computer program, frequencies, means and chi-square values were calculated.

RESULTS:

The study includes fifty samples 40 (80%) samples were malignant tumor and 10(20%) samples were benign tumor. Table (1). Out of forty samples graded, 9(22.5%) samples were well differentiated tumors, 24 (60%) samples were moderately differentiated tumors and 7(17.5%) samples were poorly differentiated tumors. Table (2) 21(42%) of patients were male, while 29(58%) patients were female. Table (3) The malignant tumor showed 23(57.5%) negative metastasis while 17(42.5%) positive metastasis. Table (4) The age of study subjects revealed that less than 60 years were 42 (84%) patients, and more than 60 years were 8(16%) patients. Table (5) Malignant colorectal tumors revealed positive expression of survivin in 8(16%) samples and negative in 32(64%) samples, while all benign tumors showed no in positive result with insignificant association (P= 0.123) Table (6). Histological differentiation and survivin positive expression revealed that 1/9sample was well differentiated tumor, 7/24 samples were moderate differentiated tumor and 0/7 sample was poor differentiated tumor with no significant statistical association (P=0.178) Table (7) The relation between metastasis and survivin expression showed 23(57.5%) samples were negative metastasis and 17(42.5%) samples were positive metastasis with no significant statistical association (P=0.201) Table (8).

Figures (1): showed immunohistochemical staining of survivin in paraffin-embedded tissues.

Table (1): Distribution of sample among study population:

Histological diagnosis	Frequency	Percent
Malignant	40	80.0%
Benign	10	20.0%
Total	50	100.0%

Table (2): Grade of malignant sample:

Tumors grades	Frequency	Percent
Well differentiated tumor	9	22.5%
Moderately differentiated tumor	24	60.0%
Poorly differentiated tumor	7	17.5%
Total	40	100.0%

Table (3): Distribution of gender among study population

Gender	Frequency	Percent
Male	21	42.0%
Female	29	58.0%
Total	50	100.0%

Table (4): Metastasis of malignant tumor:

Metastasis	Frequency	Percent
Negative	23	57.5%
Positive	17	42.5%
Total	40	100.0%

Table (5): Distribution of age among study population:

Age group	Frequency	Percent
Less than 60 years	42	84.0%
More than 60 years	8	16.0%
Total	50	100.0%

Table (6): Relation between histopathological diagnosis and survivin expressions

Histological diagnosis	Survivn expression		Total
	Positive	Negative	
Malignant	8	32	40
Benign	0	10	10
Total	8	42	50

P=0.123

Table (7): Relation between tumor grade and survivin expression

Tumor grade	Survivn expression		Total
	Positive	Negative	
Well differentiate	1	8	9

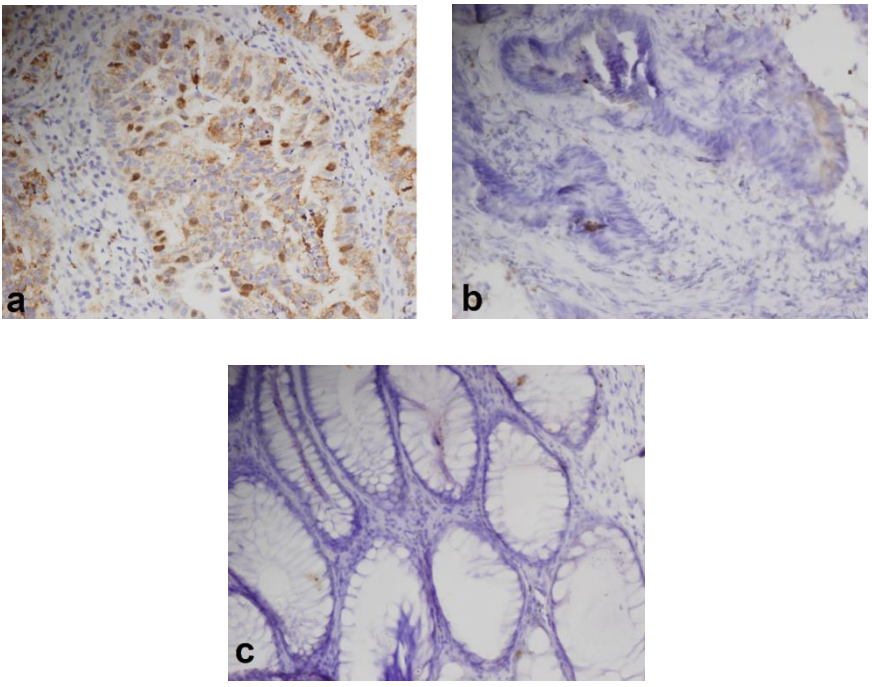
Moderate differentiate	7	17	24
Poor differentiate	0	7	7
Total	8	32	40

P=0.178

Table (8): Relation between tumor metastasis and survivin expression

Tumor metastasis	Survivin expression		Total
	Positive	Negative	
Negative	3	20	23
Positive	5	12	17
Total	8	32	40

P=0.201



Figures (1): Immunohistochemical staining of survivin in paraffin-embedded tissues:

a: Nuclear and cytoplasmic of survivin positve in colonic carcinoma.

b: Nuclear and cytoplasmic negative immunostaing in colonic carcinoma.

c: Nuclear and cytoplasmic negative immunostaing of survivin in benign hyperplastic polyps**.**

DISCUSSION:

Colorectal cancer is the second leading cause of cancer related mortality in western countries. Tumor kinetic studies suggest that tumor growth does not only result from increased rates of cell proliferation but also from decreased rates of apoptosis [18].

The present study includes 50 samples previously diagnosed as colorectal tumor, 40(80%) of them were malignant adenocarcinoma and 10(20%) were benign tumor. In current work the age group showed less than 60 years were 42(84%) patients while more than 60 years were 8(16%) patients.

In our work, study according to differentiation of malignant tumor, moderately differentiation is predominant. In accordance with our result, the prevalence of moderately differentiated adenocarcinoma is supported by STELIAN Ş.M et al., [19] which they reported more than half of the tumors were classified as moderately differentiated adenocarcinoma.

In the current work, malignant tumor showed 23(57.5%) negative metastasis while 17(42.5%) showed positive metastasis.

In our study we found that there is no statistical significant association between survivin expression and histological diagnosis,tumor differentiation and tumor metastasis, and this in accordance with the result reported by Kawaski et al.,[20]. andSarela et al.,[21][22]. In which they reported that survivin expression increase during development of colorectal tumorigenesis but no correlation was found with clinicopathological features.

CONCLUSION:

The study concluded that the survivin expression in colorectal carcinoma was not correlated with histological diagnosis, tumor differentiation and tumor metastasis.

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