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ORIGINAL ARTICLE

Immunohistochemical Expression of Survivin and MUC5AC in Colorectal Cancer

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ABSTRACT

Keyword: Colorectal cancer, Survivin, MUC5AC, IHC

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Background: Colorectal cancer (CRC) is the third most frequently malignancy and the second leading cause of cancer-related deaths globally. Among emerging biomarkers, Survivin, an inhibitor of apoptosis, and MUC5AC, a mucin glycoprotein, have garnered attention due to their involvement in cell division, apoptosis regulation, and tumor progression. Their expression may hold potential diagnostic and prognostic value in CRC. **Objectives** to assess the expression of Survivin and MUC5AC in CRC tissues compared to normal colonic mucosa and explore their correlation with various clinico-pathological features, including tumor size, histological grade, lymph node metastasis (LNM), tumor budding, and lymphovascular invasion (LVI). and prognostic relevance in CRC patients. **Material and methods:** A total of 102 paraffin-embedded tissue samples (51 CRC, 51 normal mucosa) were evaluated using immunohistochemistry (IHC). An immunoreactivity score (IRS) was used, based on staining intensity and the percentage of positively stained cells. **Results:** revealed significantly elevated expression levels of Survivin and MUC5AC in CRC tissues ($p < 0.001$). Both markers were strongly associated with LNM and LVI, while Survivin alone correlated with tumor budding. Their co-expression was statistically significant, supporting their potential as diagnostic and prognostic biomarkers in CRC. **Conclusion:** Survivin and MUC5AC are promising biomarkers for CRC diagnosis and prognosis, showing strong associations with aggressive pathological features and potential clinical utility.

INTRODUCTION

By 2035, the incidence of colorectal cancer is predicted to increase by 60%, despite advancements in diagnostic techniques and preventative healthcare practices **[1].**

In 2020, there were 5231 (3.9%) new instances of colorectal cancer in Egypt, making it the sixth most common disease in both men and women. Similarly, CRC accounted for 2852 (0.2%) of all cancer-related fatalities, placing it eighth overall. Additionally, there have been some specific advancements in the screening of colorectal cancer **[2].**

Survivin belongs to the family of proteins that suppress apoptosis. In order to control chromosome separation and cell division, Survivin can function as a subunit of the chromosomal

passenger complex (CPC) and guide its other members, including Aurora-B, borealin, and the inner centromere protein **[3].** In most cancer types, poor outcomes are linked to high levels of Survivin expression **[4].** Note that Survivin has a variety of functions, including regulating apoptosis and cell proliferation. In addition to preventing cell death and reducing apoptosis, Survivin's presence in tumors is linked to chemotherapy resistance and the aggressive nature of malignancies **[5].**

Another potential biomarker in CRC is MUC5AC which is encoded on chromosome (11p15.5) **[6].** The stomach, lungs, ear, conjunctiva, nasopharynx, and gallbladder are the only organs that normally express the secretory mucin MUC5AC **[7].**

Tumor cell migration, invasion, adhesion, metastasis, and proliferation have all been linked to differential mucin expression **[8].** It is well known that the malignant behavior of cancer cells is caused by tissue-specific mucin genes and mucin carbohydrate antigens that alter in various forms of carcinoma **[9].** TNM (tumor, node, metastasis) categorization is used to stage the tumor in colorectal cancer (CRC), as is the case with all cancer types, in order to make basic therapeutic decisions. Additional immunohistochemistry (IHC) and genetic testing are used to determine the patients' eventual course of treatment, even if they share the same clinical diagnosis **[10].** The patients' treatment procedures have benefited immensely from the discovery of these extra prognostic indicators. Additionally, by examining the expression levels of different materials using IHC examination, the association with prognosis can be ascertained **[11].** Researchers have been looking for certain biomarkers that may be used to diagnose and track the effectiveness of treatment for people with colorectal cancer. But as of yet, no such indexes have been discovered **[12].**

AIMS OF THE WORK:

In the current study we aimed to a. analyze the expression of Survivin and MUC5AC in CRC patients in comparison with normal colonic mucosa, b. to study the association between Survivin and MUC5AC and clinico-pathological feature of CRC, c. to correlate the expression of Survivin and MUC5AC expression in CRC patients and their relation to prognostic parameters of the tumor.

MATERIAL AND METHODS:

This case-control study involved 102 formalin-fixed, paraffin-embedded tissue samples, comprising 51 colorectal cancer specimens and 51 matched normal colonic mucosae from the same patients. These instances were chosen from colorectal cancer patients who underwent surgical procedures at the Oncology Surgical Department. The specimens were submitted to the Pathology Lab of Aswan University Hospital and Aswan Oncology Center from January 2018 to September 2023.

The investigation encompassed specimens from radical right and left hemicolectomy, abdominoperineal resection, and low anterior resection. Cases involving colonoscopic and/or rectal biopsy, prior irradiation or chemotherapy, extensive necrosis and/or fibrosis, or positive/close margins were eliminated from this investigation. Clinical data were extracted from pathology reports, encompassing patient age, sex, tumor location, dimensions, and surgical type. Three serial sections from each tissue block were prepared at a thickness of 4 microns for the following purposes: One section was stained with

standard Hematoxylin and Eosin (H&E) for histological assessment, while the other two sections were immune -stained with Survivin and MUC5AC antibodies, respectively.

All H&E -stained sections were analyzed with a light microscope to determine histological type, grade, tumor invasion depth, lymphovascular invasion (LVI), tumor budding, lymph node metastasis (LNM), perineural invasion (PNI), and staging of colorectal cancer (CRC).

For IHC analysis, 4µm tissue sections were incubated with primary monoclonal antibodies: Survivin (Catalog; Cat#MA5-17035, Thermo Fisher Scientific) at a dilution of 1:500 and MUC5AC (Cat# MA1-21907, Thermo Fisher Scientific) at a dilution of 1:100. The negative control was established by omitting the main antibody, whilst stomach tissue functioned as a positive control. The streptavidin-biotin amplification technique was employed for immunostaining.

Tissue sections were affixed to positively charged slides, subjected to overnight deparaffinization in xylene, and subsequently rehydrated through a gradient of decreasing ethanol concentrations (95%, 85%, 70%). Subsequent to rinsing with tap water, they were rinsed with phosphate-buffered saline (PBS) at a pH of 7.2-7.6. A single drop of primary antibody was administered to tissue sections and incubated at ambient temperature for 60 minutes. Subsequently, a linking solution was applied for 15 minutes, after which rinse with PBS was conducted. The avidin-biotin complex (ABC) solution was administered for 15 minutes, succeeded by further washes with PBS. A chromogen was then introduced to interact with alkaline phosphatase, resulting in a colorimetric reaction. Tissue sections were rinsed with tap water, dehydrated using progressive concentrations of alcohol (70%, 85%, and 95%), and readied for assessment.

The immunoreactivity score (IRS) was evaluated based on the percentage of positive cells and the intensity of staining. The proportion of positive cells was assessed on a scale from (0) 0% to (4) 100% positive cells, while staining intensity was evaluated from none (0) to intense (3). The IRS was determined by multiplying the percentage score by the intensity score, resulting in the following categories: 0-1 (negative), 2-3 (mild), 4-8 (moderate), and 9-12 (strong) [12].

The results were analyzed utilizing IBM SPSS software (version 20.0, Armonk, NY: IBM Corp). Qualitative data were expressed as numerical values and percentages. The Kolmogorov-Smirnov test was employed to evaluate the normality of data distribution. Quantitative data were characterized by the range (minimum and maximum), mean ± standard deviation (SD), median, and interquartile range (IQR). The Wilcoxon signed-rank test was employed to compare irregularly distributed quantitative variables across two eras. The Chi-square test was utilized to compare categorical data across various groups. Monte Carlo adjustment was used to the Chi-square test if over 20% of the cells had an expected count of fewer than 5. The Student's t-test was employed to compare two groups of normally distributed quantitative data. The Mann-Whitney test was utilized for group analysis of abnormally distributed quantitative data. Statistical significance was defined as $p < 0.05$, extremely significant as $p < 0.01$, and non-significant as $p > 0.05$.

Approval was secured from the Ethics of Scientific Research Committee at the Faculty of Medicine, Aswan University, and the privacy and confidentiality of all acquired information were maintained., IRB NUM: Asw. Uni./488/10/20

RESULTS:

This study was conducted in tissue samples of 51 patients of CRC. The mean age of studied cases was 57 years. More than half of our participants (58%) were males, Specimens

were left sided in 60.8% of cases, rectal specimens were 27.5% of cases, and right side of the colon was the least common affected site (11.8%). It was found that 54.9% of cases had tumor size more than 5cm. The majority of the studied cases were diagnosed as conventional adenocarcinoma 48/51 (94.1%), and most of them 48/51 (82.4%) were Grade II carcinomas.

LNM was positive in 27/51 (52.9%), LVI was detected in 29/51 (56.9%), while PNI was found in 11/51 (21.6%) of cases and tumor stage II and III were the most frequent stages. The 51 normal colonic mucosae from the same patient were obtained for comparison.

Survivin expression was positive in 27/51 (52.9%) of CRC cases, while no evidence of expression in normal colonic mucosa 0/51 (100%) of cases. Significant statistical difference was detected when comparing Survivin expression in CRC and normal colonic mucosae ($p<0.001$) as shown in table (1), figure (1-3)

Table (1): Comparison between Survivin Expression in CRC and Normal Colonic Mucosae

Survivin expression	CRC (n=51)		Normal colonic (n=51)		χ^2	p
	No.	%	No.	%		
Negative	24	47.1	51	100.0	36.720*	<0.001*
Positive	27	52.9	0	0.0		

χ^2: **Chi square test**
p: p value for comparing between CRC and Normal colonic tissue
*: Statistically significant at $p \leq 0.05$

Comparing the IRS of Survivin with the clinco- pathological variables showed that there was a statistically significant association between Survivin expression and both LNM and LVI ($p<0.001$ for each). There was a Significant positive association between Survivin expression and tumor budding ($p = 0.028$). However, no significant correlation could be detected between Survivin expression and other variables including patient' age and sex, tumor size, grade or PNI (table 2).

Table (2): Relation between IRS of Survivin and clinico-pathological Studied Parameters

	IRS of Survivin								Test of sig.	p
	Negative (0 – 1) (n = 24)		Mild (2 – 3) (n = 6)		Moderate (4 – 8) (n = 12)		Strong (9 – 12) (n = 9)			
	No.	%	No.	%	No.	%	No.	%		
Sex										
Male	13	54.2	1	16.7	9	75.0	7	77.8	χ^2=	MCp=
Female	11	45.8	5	83.3	3	25.0	2	22.2	6.764	0.071
Age (years)										
Mean ± SD.	57.21 ± 10.84		63.33 ± 5.92		54.83 ± 8.49		57.67 ± 11.86			
Median (Min. – Max.)	60.0 (32.0 – 71.0)		63.50 (53.0 – 70.0)		55.0 (42.0 – 70.0)		62.0 (38.0 – 73.0)		F=0.953	0.423

Site										
Left colonic specimen	12	50.0	3	50.0	8	66.7	8	88.9	χ^2= 7.396	^{MC}p= 0.232
Right colonic specimen	4	16.7	1	16.7	0	0.0	1	11.1		
Rectal specimen	8	33.3	2	33.3	4	33.3	0	0.0		
Diagnosis										
Invasive adenocarcinoma	23	95.8	5	83.3	11	91.7	9	100.0	χ^2= 2.426	^{MC}p= 0.505
Mucinous carcinoma	1	4.2	1	16.7	1	8.3	0	0.0		
Grade										
I	2	8.3	0	0	2	16.7	0	0.0	χ^2= 4.905	^{MC}p= 0.491
II	21	87.5	5	83.3	9	75.0	7	77.8		
III	1	4.2	1	16.7	1	8.3	2	22.2		
Margin (Free)	24	100.0	6	100.0	12	100.0	9	100.0	–	–
Lymph node metastasis (LNM)										
Negative	18	75.0	4	66.7	2	16.7	0	0.0	χ^2= 21.655*	^{MC}p <0.001*
Positive	6	25.0	2	33.3	10	83.3	9	100.0		
T										
T1	1	4.2	1	16.7	0	0.0	0	0.0	χ^2= 6.322	^{MC}p= 0.717
T2	10	41.7	1	16.7	4	33.3	2	22.2		
T3	11	45.8	4	66.7	7	58.3	5	55.6		
T4	2	8.3	0	0.0	1	8.3	2	22.2		
N										
N0	18	75.0	4	66.7	2	16.7	0	0.0	χ^2= 29.376*	^{MC}p <0.001*
N1	4	16.7	0	0.0	2	16.7	1	11.1		
N2	2	8.3	1	16.7	7	58.3	7	77.8		
N3	0	0.0	1	16.7	1	8.3	1	11.1		
Tumor size										
≤5	13	54.2	3	50.0	5	41.7	2	22.2	χ^2=2.812	^{MC}p= 0.437
>5	11	45.8	3	50.0	7	58.3	7	77.8		
Mean ± SD.	4.72 ± 1.62		5.12 ± 1.60		5.43 ± 1.86		6.76 ± 1.72		H=7.379	0.061
Median (Min. – Max.)	4.75 (2.30 – 8.0)		5.50 (2.50 – 7.0)		5.80 (2.20 – 9.0)		7.50 (4.0 – 9.0)			
LVI										
No	18	75.0	0	0.0	3	25.0	1	11.1	χ^2= 19.543	^{MC}p <0.001*
Yes	6	25.0	6	100.0	9	75.0	8	88.9		
Perineural invasion (PNI)										
No	17	70.8	6	100.0	9	75.0	8	88.9	χ^2= 2.579	^{MC}p= 0.465
Yes	7	29.2	0	0.0	3	25.0	1	11.1		
Tumor budding										
No	23	95.8	4	66.7	8	66.7	6	66.7	χ^2= 7.808*	^{MC}p= 0.028*
Yes	1	4.2	2	33.3	4	33.3	3	33.3		

SD: **Standard deviation** χ^2: **Chi square test** MC: **Monte Carlo**
t: Student t-test **U: Mann Whitney test**
p: p value for comparing between different categories
*: Statistically significant at $p \leq 0.05$

High statistically significant difference was detected in the expression of MUC5AC between CRC and normal colonic mucosal tissue (p<0.001). All cases of normal colonic mucosa showed negative MUC5AC expression, while more than half of cases 31/51 (60.8%) of CRC showed highly positive MUC5AC expression as shown in table (3), figure (4, 5).

Table (3): Comparison between CRC and Normal colonic mucoase regarding to MUC5AC expression

MUC5AC expression	CRC (n = 51)		Normal colonic tissue (n = 51)		χ^2	P
	No.	%	No.	%		
Negative	20	39.2	51	100.0	44.535*	<0.001*
Positive	31	60.8	0	0.0		

χ^2: **Chi square test**
p: p value for comparing between CRC and Normal colonic tissue
*: Statistically significant at $p \leq 0.05$

As regard to the relation between MUC5AC IRS and clinic- pathological data, significant statistical association was found between the IRS of MUC5AC and LNM (p <0.001) and LVI (p <0.017), while no significant association was found between the IRS of MUC5AC and other variables (table 4).

Table (4): Relation between the IRS of MUC5AC and clinic-pathological variables

	Intensity of MUC5AC								Test of sig.	p
	Negative (0 – 1) (n = 20)		Mild (2 – 3) (n = 9)		Moderate (4 – 8) (n = 15)		Strong (9 – 12) (n =7)			
	No.	%	No.	%	No.	%	No.	%		
Sex										
Male	11	55.0	6	66.7	8	53.3	5	71	χ^2=	^{MC}p=
Female	9	45.0	3	33.3	7	46.7	2	28.6	1.015	0.860

Age (years)										
Mean ± SD.	57.70 ± 11.77		54.56 ± 10.38		58.0 ± 8.48		59.29 ± 8.96		F=0.329	0.804
Median (Min. – Max.)	61.50 (32.0 – 71.0)		53.0 (38.0 – 67.0)		59.0 (42.0 – 70.0)		61.0 (45.0 – 73.0)			
Site										
Left colonic specimen	11	55.0	7	77.8	9	60.0	4	57.1	χ^2= 9.287	^{MC}p= 0.114
Right colonic specimen	2	10.0	2	22.2	0	0.0	2	28.6		
Rectal specimen	7	35.0	0	0.0	6	40.0	1	14.3		
Diagnosis										
Invasive adenocarcinoma	18	90.0	9	100.0	14	93.3	7	100.0	χ^2= 1.230	^{MC}p= 1.000
Mucinous carcinoma	2	10.0	0	0.0	1	6.7	0	0.0		
Grade										
I	2	10.0	0	0.0	2	13.3	0	0.0	χ^2= 4.210	^{MC}p= 0.633
II	17	85.0	7	77.8	11	73.3	7	100.0		
III	1	5.0	2	22.2	2	13.3	0	0.0		
Margin (Free)	20	100.0	9	100.0	15	100.0	7	100.0	–	–
Lymph node metastasis (LNM)										
Negative	17	85.0	0	0.0	6	40.0	1	14.3	χ^2= 23.869*	^{MC}p <0.001*
Positive	3	15.0	9	100.0	9	60.0	6	85.7		
T										
T1	0	0.0	1	11.1	1	6.7	0	0.0	χ^2= 6.761	^{MC}p= 0.667
T2	7	35.0	1	11.1	7	46.7	2	28.6		
T3	11	55.0	6	66.7	6	40.0	4	57.1		
T4	2	10.0	1	11.1	1	6.7	1	14.3		
N										
N0	17	85.0	0	0.0	6	40.0	1	14.3	χ^2= 32.489*	^{MC}p <0.001*
N1	2	10.0	2	22.2	1	6.7	2	28.6		
N2	0	0.0	6	66.7	8	53.3	3	42.9		
N3	1	5.0	1	11.1	0	0.0	1	14.3		
Tumor size										
≤5	10	50.0	2	22.2	9	60.0	2	28.6	χ^2= 4.039	^{MC}p= 0.255
>5	10	50.0	7	77.8	6	40.0	5	71.4		
Mean ± SD.	4.84 ± 1.36		6.63 ± 2.05		4.77 ± 1.59		5.97 ± 2.26		H=8.025*	0.046*
Median (Min. – Max.)	5.15 (2.30 – 7.40)		7.50 (2.50 – 9.0)		5.0 (2.50 – 8.0)		6.10 (2.20 – 9.0)			
LVI										
No	14	70.0	2	22.2	5	33.3	1	14.3	χ^2= 9.946*	^{MC}p= 0.017*
Yes	6	30.0	7	77.8	10	66.7	6	85.7		
Perineural invasion (PNI)										
No	15	75.0	6	66.7	13	86.7	6	85.7	χ^2= 1.716	^{MC}p= 0.683
Yes	5	25.0	3	33.3	2	13.3	1	14.3		

Tumor budding										
No	18	90.0	6	66.7	11	73.3	6	85.7	χ^2=	^{MC}p=
Yes	2	10.0	3	33.3	4	26.7	1	14.3	2.970	0.447

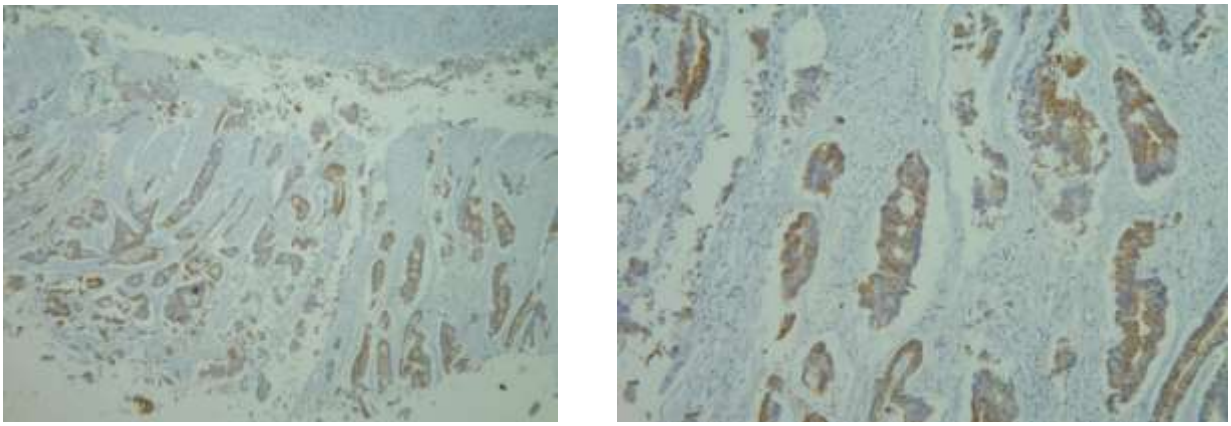
SD: **Standard deviation** χ^2: **Chi square test** MC: **Monte Carlo**
t: Student t-test **U: Mann Whitney test**
p: p value for comparing between different categories
*: Statistically significant at $p \leq 0.05$

Significant statistical association was detected between Survivin and MUC5AC expressions in CRC cases (p<0.001). Positive expression of Survivin and MUC5AC in CRC cases was found in 25/27 (92.6%) of cases as shown in table (5)

Table (5): Relation between Survivin and MUC5AC expressions in CRC cases

MUC5AC expression	**Survivin expression**				χ^2	**P**
	Negative (N = 24)		**Positive (N = 27)**			
	No.	**%**	**No.**	**%**		
Negative	18	75.0	2	7.4	24.353*	<0.001*
Positive	6	25.0	25	92.6		

χ^2**:** Chi Square Test



A: **B**:

Figure (1): Moderate positive Survivin expression (IRS 8) in tumor cells of CRC (A. x40, B, x100)

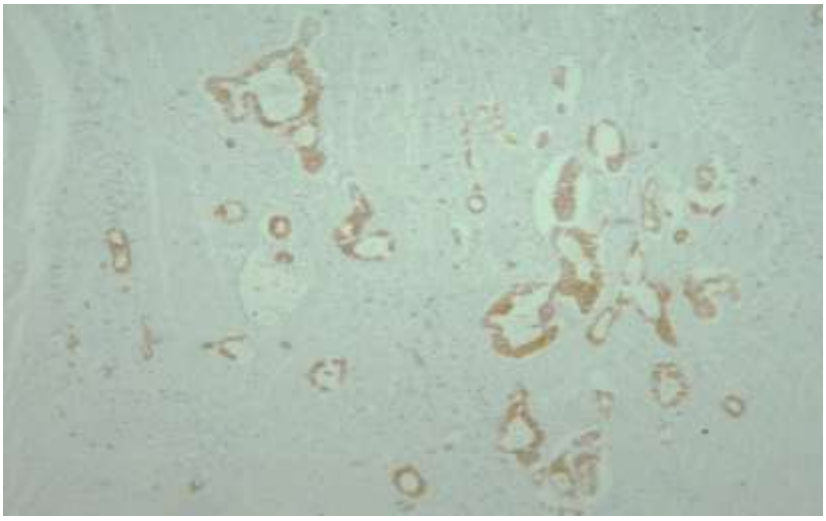
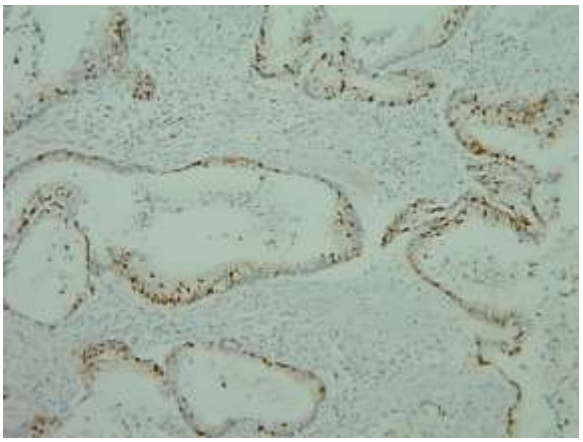


Figure (2): Strong positive Survivin expression (IRS 9) in tumor cells of CRC (x40)



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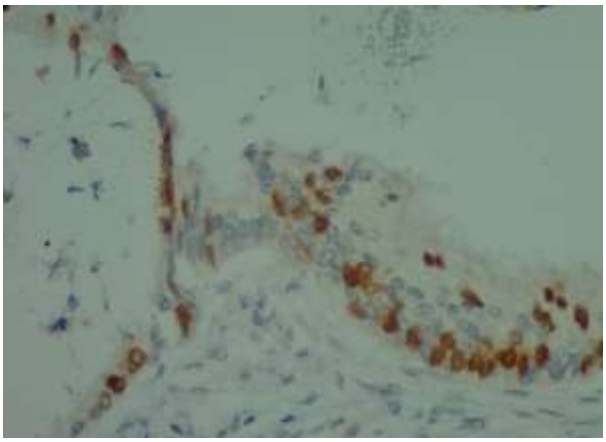
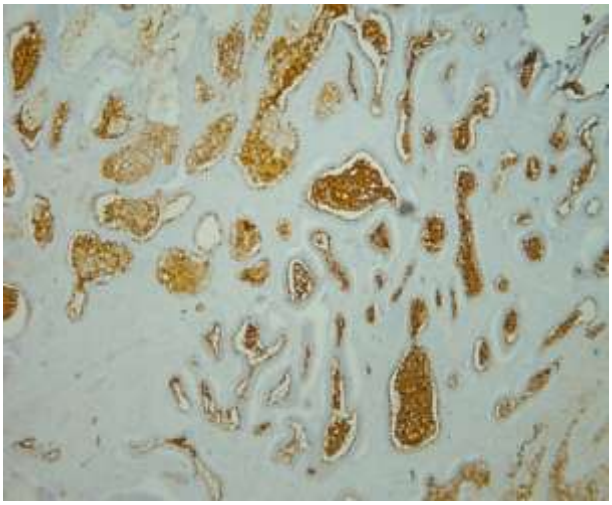


B:

Figure (3): CRC showing strong positive Survivin expression (IRS 12) in tumor cells (A. x40, B. x100)



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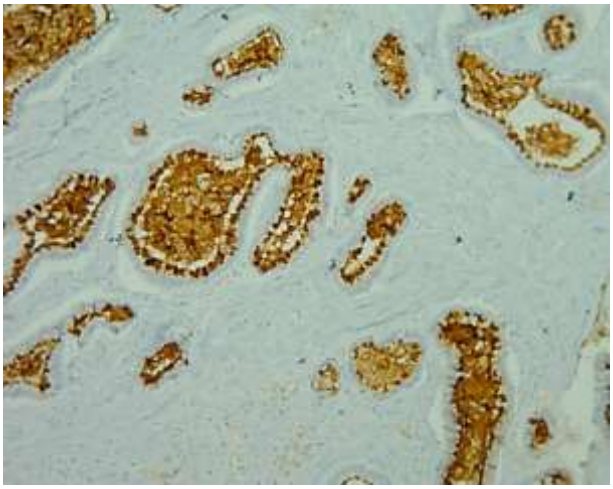

B:

Figure (4): CRC showing strong positive MUC5AC expression (IRS12) in tumor cells (A. x40, B. x100)

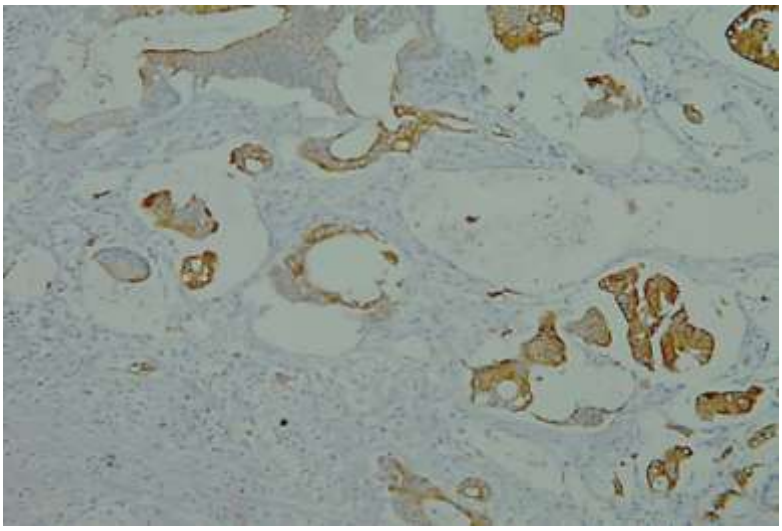

Figure (5): CRC showing strong positive MUC5AC expression (IRS 12) in tumor cells (x100).

DISCUSSION:

Survivin plays a crucial role in cancer development and is involved in tumor cell resistance to radiation and chemotherapy. Mucins are high-molecular-weight glycoproteins. Over 20 mucin types have been identified and categorized into two classes according to their structure and function, playing a role in neoplasm growth **[13].** To our knowledge, no research have yet examined the link between Survivin and MUC5AC expressions in colorectal cancer among Egyptian patients.

This study aims to assess the immunohistochemical expressions of Survivin and MUC5AC and their relationship with colorectal cancer (CRC) in comparison to healthy colonic mucosa, as well as the correlation of their expression with clinico-pathological characteristics in CRC. This study revealed a substantial statistical difference in Survivin expression between colorectal cancer (CRC) and normal colonic mucosae ($p<0.001$). Survivin was expressed in 52.9% of CRC patients, but no expression was seen in normal colonic mucosae. This outcome aligned with the work of Al-Muttairi et al., which utilized Chromogenic In-Situ Hybridization (CISH) and demonstrated that the percentage of positive Survivin expression in colorectal cancer (CRC) was 63.3%, in contrast to 55% in benign equivalents. A significant proportion of cases in CRC exhibited the Survivin gene, with a moderate score observed in 26.7% of instances. They established that Survivin was absent in normal colorectal mucosa **[14].** Jourabchin et al. discovered that the expression rate of Survivin in colonic polyps was 81.6%, whereas in colorectal cancer (CRC) it was 94.6%. Survivin expression was elevated in colorectal cancer compared to colonic polyps [15]. Additionally, Al-Maghrabi et al. discovered that Survivin expression was present in 60.8% of the colorectal cancer samples examined **[16].** The present investigation found no significant correlations between Survivin expression and patients' age, sex, tumor location, size, or grade ($p > 0.05$ for all) in the analyzed cases. The findings concurred with those of Almuttairi et al., Wang et al., and Al-Maghrabi et al., who established that there were no significant relationships between age, sex, tumor site, tumor size,

or grade in the examined CRC cases and Survivin expression **[13],[14],[16].** Conversely, Shintani et al. discovered that females had significantly elevated levels of nuclear Survivin expression compared to males ($p < 0.05$). This variation may be attributed to the utilization of a different technology; the enzyme-linked immunosorbent assay method was employed to quantify the amount of Survivin **[17].**

This study identified a statistically significant association between Survivin expression and both lymph node metastasis (LNM) and lymphovascular invasion (LVI), with p-values less than 0.001 for each. This finding aligns with the work of Al-Maghrabi et al., who demonstrated a statistically significant association between positive Survivin immunostaining and both LNM and LVI **[16].**

Consistent with Zhang et al., a statistically significant correlation was seen between Survivin expression and tumor budding (p=0.028) **[18].**

This investigation, in agreement with Al-Muttairi et al., revealed no significant connection between Survivin expression and PNI **[14].**

This study identified a substantial statistical difference in MUC5AC expression between colorectal cancer (CRC) and normal colonic mucosae (p=<0.001). All instances of normal colonic mucosa exhibited negative MUC5AC expression, but over half of colorectal cancer cases (60.8%) demonstrated elevated MUC5AC expression.

Moreover, Jayanth et al. discovered that none of the normal individuals exhibited MUC5AC expression, but a significant majority of the CRC cases (88%) demonstrated moderate to high MUC5AC expression, particularly in mucinous carcinomas **[19].** Moreover, Wang et al. discovered that MUC5AC was highly expressed in 28.06% of colorectal cancer cases, whereas absent in normal colon tissues **[13].**

This study found no significant correlation between MUC5AC expression and factors such as patients' age, sex, tumor location, size, or grade in the examined CRC cases. This aligns with the findings of Wang et al. and Hazgui et al., who identified no significant statistical correlation between MUC5AC expression and the patients' age, sex, tumor location, size, or grade **[13],[20].**

Walsh et al. (2013) identified a significant statistical correlation between MUC5AC expression and female sex in a prospective cohort study involving 41,514 colorectal cancer (CRC) cases, demonstrating that MUC5AC-positive CRC was more frequently located proximally. This disparity may be attributed to the substantial sample size of 702 cases and differing geographic factors **[21].** The research by Kesari et al. shown a strong correlation between MUC5AC expression and higher grades of colorectal cancer (p= 0.006) **[22].**

The current investigation identified a statistically significant correlation between MUC5AC expression and lymph node metastasis (LNM). This discovery corresponds with the research conducted by Wang et al., which indicated elevated MUC5AC expression in colorectal cancer cases exhibiting lymph node metastasis. Nonetheless, Hazgui et al. discovered no significant link between MUC5AC expression and lymph node metastasis (LNM) in their investigation, which employed both immunohistochemistry (IHC) and reverse-transcription polymerase chain reaction (RT-PCR) to assess MUC5AC expression **[13],[20].**

Moreover, in alignment with Wang et al., the present investigation revealed a statistically significant correlation between MUC5AC expression and LVI. Conversely, Hazgui et al. could not identify a significant link between MUC5AC expression and PNI in their research **[13, 20].**

The current investigation identified a strong positive statistical connection between the expressions of Survivin and MUC5AC in colorectal cancer cases. Moreover, the positive association between Survivin and MUC5AC in colorectal cancer cases was 92.6%. This data

aligns with Wang et al., who identified a substantial positive connection between Survivin and MUC5AC expression in colorectal cancer cases **[13].**

CONCLUSION:

In conclusion, elevated expressions of Survivin and MUC5AC were observed in colorectal cancer cases, but no expression of either was detected in normal colonic mucosa. Furthermore, instances of CRC exhibiting elevated levels of Survivin and MUC5AC expression revealed markedly higher rates of LVI and LNM. Moreover, there were notable correlations between Survivin expression and tumor sprouting. The findings indicate that elevated levels of Survivin and/or MUC5AC may correlate with tumor growth and adverse prognostic indicators (LVI, tumor budding, and LNM) in colorectal cancer, and their expression could facilitate early detection of the disease.

RECOMMENDATION:

- Further studies with a larger number of patients, and in multi-centers are necessary to explore the role of Survivin and MUC5AC in CRC patients.
- Studying the correlation between studied markers and other prognostic parameters such as overall survival and disease-free survival rate.

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