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Immunohistochemical Expression of Galectin-3 in Colorectal Carcinoma

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Abstract: Colorectal cancer is one of the leading causes of cancer death in both developed and developing nations. Galectin-3 is a β-galactoside-binding protein, whose expression has been correlated with progressionand metastasis in colon cancer. We aimed in this study to evaluate the immunohistochemical expression of Galectin-3 in colorectal carcinoma and its relation with various clinicopathologic variables. Sixty paraffin embedded tissue blocks from colectomy specimens of colorectal carcinoma obtained from pathology department of Cairo University were studied immunohistochemically for Galectin-3 expression. *Results showed that:* Galectin-3 expression was positive in 81.7% of total colorectal carcinoma studied cases. There was no statistical relationship detected between galectin-3 expression regarding age, sex, tumor gross pattern and tumor size, the depth of tumor invasion, lymph node metastasis, distant metastasis and vascular invasion. Statistically significant relationships were detected between galectin-3 expression regarding tumor site (rectum predominating; P value=0.038), histological type (89.8% of conventional adenocarcinoma cases were galectin-3 positive while 63.6% of mucoid adenocarcinoma cases were galectin-3 negative) (P value <0.001), the histological grade(100% of grade I, 93.75% of grade II and 57.2% of grade III were galectin-3 positive) (P value=0.002) and modified Dukes' stage (98% of positive galectin-3 expression cases were modified Dukes' stage B&C) (P value=0.01789). *In Conclusion& Recommendations:* Therefore, it is suggested that galectin-3 may be used as a prognostic marker to predict poor outcome in patients with colorectal carcinomaand it will be necessary to carry out similar studies on a larger sample size.

Key words: Colorectal Carcinoma · Galectin-3

INTROUDICTION

Colorectal cancer (CRC) is the most common cancer worldwide [1], being the fourth most prevalent malignancy [2]. In Egypt; it occupied the first rank among digestive system's malignancies representing 15.78% and the fifth rank among all total cancers representing 4.34% [3].

The galectins are a family of carbohydrate-binding proteins characterized by domains and their affinity for β-galactosidecontaining glycoconjugates. An important member of this family is galectin-3 which is broadly expressed in normal and neoplastic cells and has been implicated in diverse biologicalfunctions including cell growth, differentiation, adhesion, apoptosis, malignant transformation and RNA processing [4, 5]. It provides tumor cells with anti-apoptotic activities, which are thought to be critical for anchorage-independent cell survival in the circulation that takes place during dissemination [6, 7] and its expression is correlated with cancer aggressiveness and metastasis [8].

Some studies have shown that the immunohistochemical expression of galectin-3(Gal-3) is uniformly elevated with neoplastic progression in certain malignancies, including gastric [9], anaplastic large-cell lymphoma [10], thyroid [11], pulmonary [12] and colon cancer [13-15].

Many authors have shown that galectin-3 can be a reliable diagnostic marker in many cancers and one of the target proteins in cancer treatment [16]. Galectin-3 expression evaluation utilizing immunohistochemistry techniques is a sensitive, specific and accurate marker in certain cancers, in addition it could be one of the target proteins of cancer treatment and targeting it could improve the efficacy of anticancer drug chemotherapy in

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several types of cancer. Because of this, galectin-3 expression in many cancer types requires further careful study [17].

The aim of this study was to evaluate the immunohistochemical expression of galectin-3 in colorectal carcinoma and its relation with various clinicopathologic variables.

MATERIAL AND METHODS

Study Group: This work included 60 cases of primary colorectal carcinomas obtained through collection of archived paraffin blocks of colectomy specimens during the period from January 2011 till March 2013, from the Pathology department, Faculty of medicine, Cairo University.

Histopathological Evaluation: Formalin fixed and paraffin embedded blocks were sectioned at 5µm thickness and examined microscopically using H&E stain to evaluate the histopathological type, grade, stage and, nodal metastasis. The tumors were staged according to two staging systems, the widely used modified Dukes' stage (Modified Astler-Coller) and American Joint Committee on Cancer (AJCC) Tumor Node Metastasis (TNM) system (2010) and graded according to the World Health Organization criteria [18-20].

Immunohistochemical Procedure: The sections were deparaffinized in xylene, then were hydrated through a series of graded alcohols (95%-70%), distilled water and phosphate buffered saline (at ph 7.5).The slides were then immersed in 10mM citrate buffer (ph. 6) and were twice pretreated by microwaving oven 800w for 4 then 8 minutes. Between each period of heating, evaporated fluid was replenished. After a 25 minute cooling period, the endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxide (H2O2) for 5 minutes. Antigen retrieval was done by immersing the slides in 10mm citrate buffer, (pH 6), for 10-20 minutes at 100°C in a microwave followed by cooling at room temperature for 20 minutes. The tissues were blocked with protein blocking reagent for 30 minutes to reduce nonspecific staining. After washing with Tris-buffered saline, the sections were incubated with the primary antibody for 1 hour at room temperature. The primary antibody was mouse monoclonal antibody, anti-Galectin-3 Clone (9C4), concentrate antibody (Snaf medical company). The sections were washed in Tris-buffer and incubated with avidin-biotin-peroxidase system (DAKO) for 30 minutes. The excess reagent was tapped off and the slides were washed with PBS and dried. Peroxidase reaction was detected by addition of diamonobenzidine tetrahydrochloride. Two or three drops of streptavidin enzyme label were placed on each slide for 30 minutes at room temperature. The excess reagent was tapped off and the slides were washed with PBS and dried.All slides were rinsed well in tap water for 5 minutes then slightly counterstained with Mayer's Hematoxylin for 1-2 minutes and dehydrated in ascending alcohol.The slides were cleared in xylene for 3 changes and then Canada balsam and cover slips were applied.

Evaluation of Expression of Galectin-3: Tumor tissue sections from each sample were examined under the microscope at high power magnification (x400) for the presence of membranous, cytoplasmic and nuclear staining of galectin-3. The signal of intensity was scored as null; weak; moderate and strong when the percentage of galectin-3 positive staining cells was as follows: 0-10%; from 10 to 25%; from 25 to 50% and > 50% respectively [21].

To avoid artificial effects, cells in areas with necrosis, poor morphology or at the margins of sections were not counted.

The positive control forgalectin-3 is conventional papillary thyroid carcinoma as it is expressed in all cases of conventional papillary thyroid carcinoma [22].

The internal control was the expression of normal colonic mucosa to galectin-3 which was strong nuclear and weak cytoplasmic expression [23].

The results of galectin-3 immunostaining in the tumors were correlated with multiple prognostic factors (age, sex, histopathological type and grade of differentiation, depth of invasion, lymph node metastasis, modified Dukes' stage, vascular invasion and distant metastasis). Correlations between the pattern of galectin-3 staining and histological type, histological grade, depth of invasion & modified Dukes' stage were also held.

Statistical Methods: Statistical analysis was performed using Statistical Package for Social Sciences, Version 17.0 (SPSS, Inc., Chicago, III., USA) for Windows. Continuous variables were analyzed as mean values ± standard deviation (SD) or median (range) as appropriate. Percentages were calculated for categorical data. For categorical variables, differences were analyzed with χ^2 (chi square) tests and Fisher's exact test when appropriate. Differences among continuous variables with normal distribution were analyzed by Student's T-test. P value of ≤ 0.05 was considered statistically significant.

RESULTS

Sixty randomly collected colorectal carcinomas were studied. The age of patients ranged from 14 to 77 years with mean age 48.2 years and median age 50 years. Histologically, 46 cases were adenocarcinomas, 12 cases were mucinous adenocarcinomas and 2 cases were signet ring cell carcinomas. The grade of differentiation in adenocarcinomas was well differentiated in 7 cases, moderately differentiated in 32 cases and poorly differentiated in 21 cases. The 12 cases of mucinous adenocarcinomas and 2 cases of signet ring cell carcinomas were considered poorly differentiated. There were 29 cases of modified Dukes' stage B (TNM stage II), 29 cases of stage C (TNM stage III) and 2 cases of stage D (TNM stage IV). The clinicopathologic characteristics of the samples are shown in Table 1.

In the present study the percentage of galectin-3 positive expression cases was 81.7% of total cases, while the percentage of galectin-3negative expression cases was 18.3%of total cases Table 2.

Regarding the pattern of staining (Table 3) in normal colonic mucosa was nuclear and weak cytoplasmic {Figure (1)} and regarding the pattern of staining in the tumorous areas was demonstrated as follows: cytoplasmic staining with membranous reinforcement was detected in 11.7% of all cases which was encountered in well differentiated tumors {Figure (2)}. 50% of all cases showed cytoplasmic and weak nuclear staining which was encountered in moderately differentiated tumors {Figure (3), (4) & (5)}. Nuclear and weak cytoplasmic staining was detected in 20% of all cases [seven cases of poorly differentiated tumors {Figure (6)} and five cases of mucoid carcinoma {Figure (7)}]. Finally, in 18.3% of all

Table 1: Clinicopathologic features of 60 colorectal (CRC) cases

Clinicopathologic features (n=60)	Number of cases	Percent
Age		
<50 years	32	53.30%
>50 years	28	46.70%
Sex		
Male	34	56.70%
Female	26	43.30%
Tumor location		
Cecum	2	3.30%
Ascending Colon	15	25%
Hepatic Flexture	3	5%
Transverse Colon	7	11.70%
Splenic Flexture	2	3.30%
Descending Colon	8	13.30%
Sigmoid Colon	6	10%
Rectum	17	28.30%
Histopathological type		
Adenocarcinoma	46	76.70%
Mucinous adenocarcinoma	12	20%
Signet ring cell carcinoma	2	3.30%
Tumor differentiation		
Well differentiated	7	11.70%
Moderately differentiated	32	53.30%
Poorly differentiated	21	35%
Modified Dukes' stage		
B	29	48.30%
C	29	48.30%
D	2	3.40%
Nodal Status	29	51.70%
Negative	31	48.30%
Positive		
Depth of tumor invasion		
T2	13	21.70%
T3	37	61.70%
T4	10	16.60%

Table 2: Galectin-3 expression in the tumor cells in the studied cases.

Galectin-3 expression			Weak	Number of cases	Percent
	Positive		Moderate	8	13.3%
	----------			17	28.4%
	No= 49	Percent= 81.7%			
	Negative		Strong	24	40%
	----------		Null	11	18.3%
	No=11	Percent=18.3%			
		Total		60	100%

Table 3: Galectin-3 expression in the tumor cells in the studied cases according to the staining pattern.

Pattern of expression		Number of cases	Percent
	Cytoplasmic and weak nuclear	30	50%
	Cytoplasmic with membranous reinforcement	7	11.7%
	Nuclear and weak cytoplasmic	12	20%
	Negative	11	18.3%
	Total	60	100%

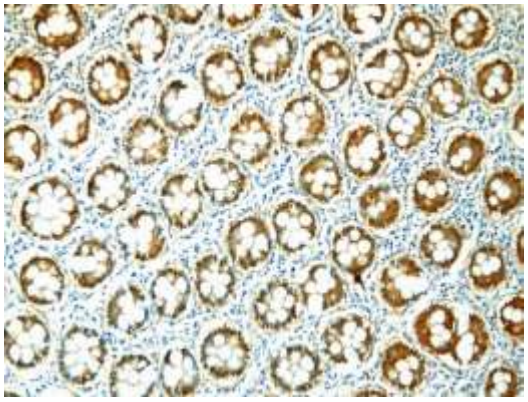

Fig 1: Nuclear and weak cytoplasmic expression of galectin-3 expression in normal colonic mucosa. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x200).

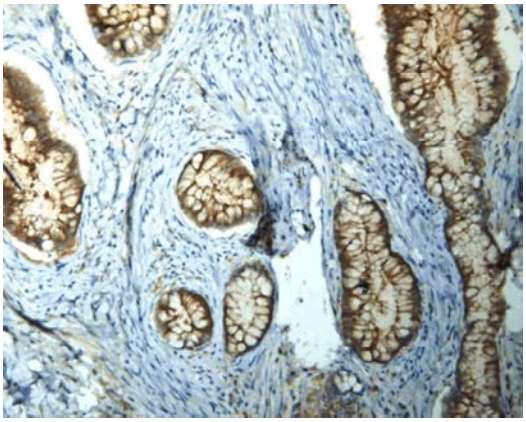

Fig 2: Cytoplasmic staining with membranous reinforcement of galectin-3 expression in well differentiated adenocarcinoma. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x200).

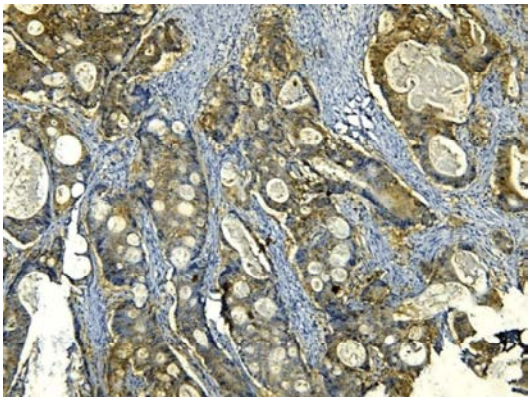

Fig 3: Weak cytoplasmic of galectin-3 expression in moderately differentiated adenocarcinoma. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x200).

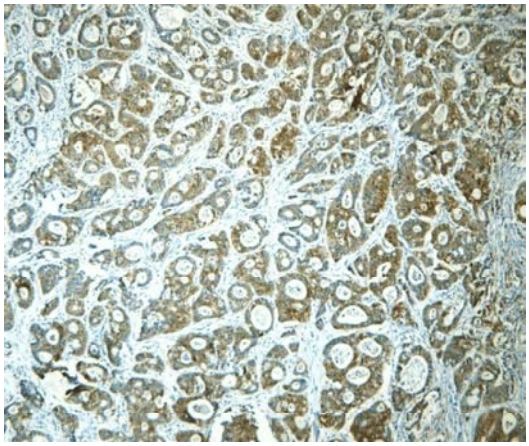

Fig 4: Moderate cytoplasmic of galectin-3 expression in moderately differentiated adenocarcinoma. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x100).

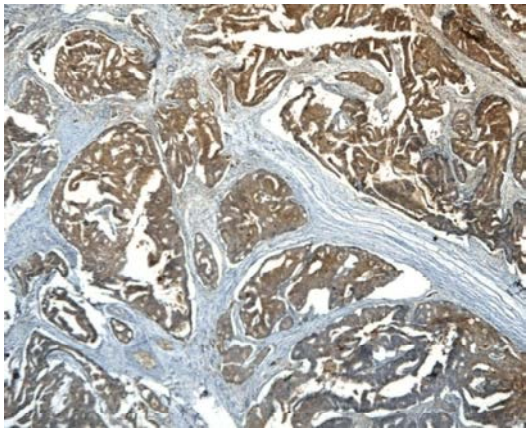

Fig 5: Strong cytoplasmic staining of galectin-3 expression in moderately differentiated adenocarcinoma. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x100).

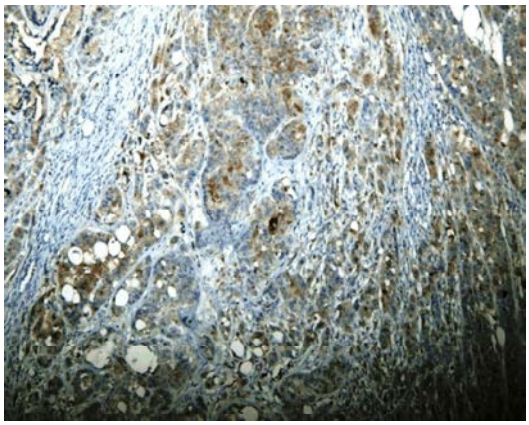

Fig 6: Poorly differentiated adenocarcinoma showing nuclear and weak cytoplasmic galectin-3. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x200).

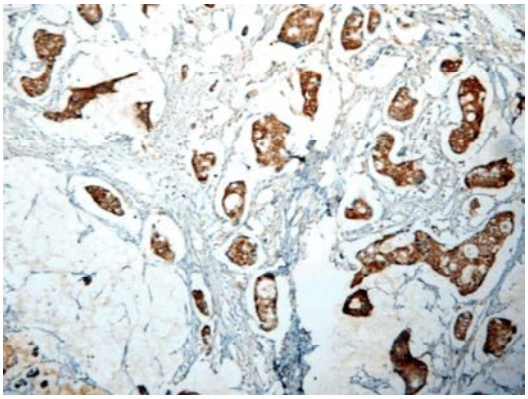

Fig 7: Positive nuclear and weak cytoplasmic galectin-3 expression in mucoid adenocarcinoma. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stainx100).

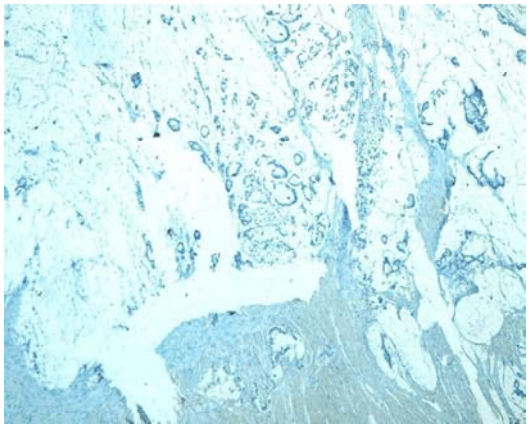

Fig 8: Negative galectin-3 expression in mucoid adenocarcinoma. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x100).

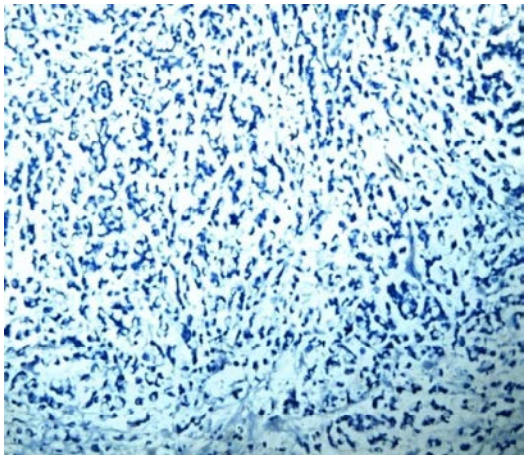

Fig 9: Signet ring carcinoma showed negative galectin-3 expression. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x100).

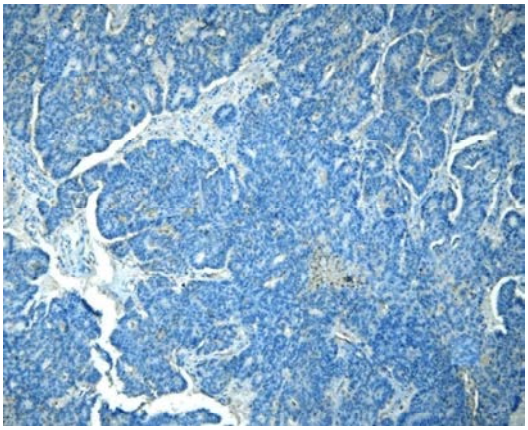

Fig 10: Negative galectin-3 expression in moderately differentiated adenocarcinoma. (Galectin-3 antibody with DAB chromogen and hematoxylin counter stain x200).

cases galectin-3 expression was negative [seven cases of mucoid carcinoma {Figure (8)}, two cases of signet ring carcinoma {Figure (9)} and two cases of moderately differentiated tumors {Figure (10)}].

There was no statistical relationship between galectin-3 expression and the age in the studied cases (P value ,=0.323).There was no statistical relationship between galectin-3 expression and the sex in the studied cases (P value =0.875). A statistically significant relationship was reported between galectin-3 expression and the tumor location, where galectin-3 expressing cases were predominantly in rectum (13 cases) (P value=0.038). A statistically significant relationship was detected between galectin-3 expression and the histological type in the studied cases where (89.8%) of conventional adenocarcinoma cases were galectin-3 positive while (63.6%) of mucoid adenocarcinoma cases were galectin-3 negative (P value <0.001).A statistically significant relationship was detected between galectin-3 expression and the histological grade in the studied cases where (100%) of well differentiated (grade I) tumors, (93.75%) of moderate differentiated (grade II) tumors and (57.2%) of poorly differentiated (grade III) tumors were galectin-3 expression positive (P value=0.002). There was no statistical relationship between galectin-3 expression and the depth of tumor invasion in the studied cases, while (55.1%) of galectin-3 positive cases were T3 (P value =0.07).There was no statistical relationship between galectin-3 expression and the lymph nodes metastasis in the studied cases (P value =0.833). There was no statistical relationship between galectin-3 expression and the distant metastasis in the studied

Table 4: Relationship of galectin-3 expression in tumor cells and otherclinicopathological variables.

	Expression of Galectin-3					
	Negative		Positive			
Variables	No=11	Percent=18.3%	No=49	Percent=81.7%	Total	P Value
Age	11	-18.30%	49	-81.70%	60 -100%	0.323
Sex						
Female	5	-45.50%	21	-42.90%	26 -43.30%	
Male	6	-54.50%	28	-57.10%	34 -56.70%	0.875
Cecum	0	0.00%	2	-4.10%	2 -3.30%	0.038
Hepatic Flexure	0	0.00%	3	-6.10%	3 -5.00%	
Left Colon	2	-18.20%	6	-12.20%	8 -13.30%	
Rectum	4	-36.40%	13	-26.50%	17 -28.30%	
Right Colon	4	-36.40%	11	-22.40%	15 -25.00%	
Sigmoid Colon	0	0.00%	6	-12.20%	6 -10.00%	
Splenic Flexure	0	0.00%	2	-4.10%	2 -3.30%	
Transverse Colon	1	-9.10%	6	-12.20%	7 -11.70%	
Histological type Adenocarcinoma	2	-18.20%	44	-89.80%	46 -76.70%	<0.001
Mucoid adenocarcinoma	7	-63.60%	5	-10.20%	12 -20%	
Signet ring cell carcinoma	2	-18.20%	0	0%	2 -3.30%	
Histological grade Well differentiated	0	0%	7	-100%	7 -11.70%	0.002
Moderately differentiated	2	-6.25%	30	-93.75%	32 -53.30%	
Poorly differentiated	9	-42.80%	12	-57.20%	21 35%))	
Depth of invasion T2	1	-9.10%	12	-24.50%	13 -21.70%	0.07
T3	10	-90.90%	27	-55.10%	37 -61.70%	
T4	0	0%	10	-20.40%	10 -16.60%	
Nodal status Negative	5	-45.50%	24	-49.00%	29 -48.30%	0.833
Positive	6	-54.50%	25	-51.00%	31 -51.70%	
Distant metastasis Negative	11	-100%	47	-95.90%	58)96.7%)	0.633
Positive	0	0%	2	-4.10%	2)3.3%)	
Modified Dukes' stage B	5	45.50%	24	49.00%	29 -48.30%	0.01789
C	5	45.50%	24	49.00%	29 -48.30%	
D	1	9.10%	1	2.00%	2)3.4%)	

cases (P value = 0.633).A statistically significant relationship was detected betweengalectin-3 expression and modified Dukes' stageamong the studied cases, where (98%) of positive galectin-3 expression cases where modified Dukes' stage B&C each was (49%) (P value=0.01789) (Table4).

No statistically significant relationship could be detected between the pattern of galectin-3 expression and the histological types. However, (65.2%) of conventional adenocarcinoma cases showed cytoplasmic and weak nuclear staining, (15.2%) of conventional adenocarcinoma cases showed cytoplasmic with membranous reinforcement, (15.2%) of conventional adenocarcinoma cases showed nuclear and weak cytoplasmic staining & (4.4%) of conventional adenocarcinoma cases showed negative staining. (41.7%) of mucoid adenocarcinoma cases showed nuclear staining and weak cytoplasmic and (58.3%) of mucoid adenocarcinoma cases showed negative staining. (100%) of signet ring carcinoma cases showed negative staining (Table 5).

Regarding the relation between the pattern of galectin-3 expression and the histological grades no statistically significant relationship could be detected. However, 100% of well differentiated cases showed cytoplasmic with membranous reinforcement staining pattern of galectin-3. (93.75%) of moderately differentiated cases showed cytoplasmic and weak nuclear staining pattern of galectin-3. (57.1%) of poorly differentiated cases showed nuclear and weak cytoplasmic staining pattern of galectin-3 & (42.9%) of poorly differentiated cases showed negative staining pattern of galectin-3 (Table 6).

No statistically significant relationship could be detected betweenthe pattern of galectin-3 expression and both the depth of tumor invasion (Table 7) and modified Dukes' stage (Table 8).

Table 5: The relationship between the pattern of galectin-3 staining and the histological types.

	Histological type			
	Adenocarcinoma (46)	Mucoid adenocarcinoma (12)	Signet ring Carcinoma (2)	P-value
Cytoplasmic and weak nuclear	30 (65.2%)	0(0%)	0(0%)	0.234608
No=30 Percent= 50%				
Cytoplasmic with membranous reinforcement	7 (15.2%)	0(0%)	0(0%)	0.448204
No=7 Percent= 11.7%				
Nuclear and weak cytoplasmic	7 (15.2%)	5(41.7%)	0(0%)	0.143932
No=12 Percent= 20%				
Negative	2 (4.4%)	7 (58.3%)	2 (100%)	0.20997
No=11 Percent= 18.3%				

Table 6: The relationship between the pattern of galectin-3 staining and the histological grades.

	Histological grade			
	GI (7)	GII (32)	GIII (21)	P-value
Cytoplasmic and weak nuclear	0 (0%)	30 (93.75%)	0(0%)	0.234608
No=30 Percent= 50%				
Cytoplasmic with membranous reinforcement	7 (100%)	0(0%)	0(0%)	0.448204
No=7 Percent= 11.7%				
Nuclear and weak cytoplasmic	0(0%)	0(0%)	12 (57.1%)	0.32332
No=12 Percent= 20%				
Negative	0(0%)	2 (6.25%)	9 (42.9%)	0.291142
No=11 Percent= 18.3%				

Table 7: The relationship between the pattern of galectin-3 staining and the depth of tumor invasion.

	Depth of tumor invasion (T)			
	T2 (13)	T3 (37)	T4 (a,b) (10)	P-value
Cytoplasmic and weak nuclear	7(53.8%)	17(45.9%)	6 (60%)	0.43929
No=30 Percent=50%				
Cytoplasmic with membranous reinforcement	4(30.7%)	3 (8.1%)	0(0%)	0.407451
No=7 Percent=11.7%				
Nuclear and weak cytoplasmic	0(0%)	8(21.6%)	4(40%)	0.22405
No=12 Percent=20%				
Negative	2 (15.5%)	9 (24.4%)	0(0%)	0.316626
No=11 Percent=18.3%				

Table 8: The relationship between the pattern of galectin-3 staining and modified Dukes' stage.

	Modified Dukes' staging			
	B (29)	C (29)	D (2)	P-value
Cytoplasmic and weak nuclear	16 (56.2%)	14(48.3%)	0(0%)	0.094731
No=30 Percent=50%				
Cytoplasmic with membranous reinforcement	6 (20.7%)	1(3.4%)	0(0%)	0.436079
No=7 Percent=11.7%				
Nuclear and weak cytoplasmic	2 (6.8%)	9(31.1%)	1(50%)	0.240909
No=12 Percent=20%				
Negative 5 (16.3%)	5(17.2%)	1(50%)	0.157648	
No=11 Percent=18.3%				

DISCUSSION

Colorectal cancer is the fourth most common cause of cancer-related mortality. There is a wide variability in the incidence rates of colorectal cancer in both developed and developing nations. It occupies the fourth rank among all types of cancers and the first gastro-intestinal cancer by organ location [24].

Early diagnosis of CRC, successful surgical treatment, better knowledge of its clinicopathological prognostic factors and response to adjuvant therapy have contributed to improved outcome in affected patients. Therefore, identification of molecular markers associated with carcinogenesis, tumor growth, invasion and metastasis has been critical to develop potential therapeutic intervention [25].

Gal-3 is a β galactoside binding, small molecular weight (about 30 kDa) protein was described as a versatile multifunctional protein involved in multiple biological processes, including cell growth, cell cycle progression, cell migration, cell adherence, proliferation, differentiation, RNA processing or negative regulation of apoptotic mechanisms and malignant transformation [26, 27].

Some authors hypothesized that there is a prognostic value of gal-3 expression in colorectal cancer as a marker of progression& metastatic potential [13, 14, 28, 29]. However, conflicting results were reported in the literature regarding the prognostic value of Gal-3 expression in colon cancer as the pattern of immunohistochemical galectin-3 expression inhuman colorectal cancer was a matter of debate because some investigators found increasing galectin-3 levels in colorectal cancer progression, whereas others did not [23, 30, 29, 31].

In the current study the percentage of galectin-3 positive expression cases was 81.7% of total cases. The negative galectin-3 expression cases represented 18.3% of total cases (Table 2). The increase of galectin-3 expression in CRC cases in the current study goes in concordance to what was reported in many studies done by Lee *et al*. [32], Ohannesian *et a*l. [33],

Schoeppner *et al*. [34], Sanjuan *et al.* [23], Endo *et al*. [15], Tsuboi *et al.* [31] and Arfaoui-Toumi *et al*. [21] that galectin-3 expression increases in colorectal cancer.

In our study strong galectin-3 expression was observed in 40% of galectin-3positive expression cases which were in preponderance but slightly lower than what was reported by *Povegliano et al.* [35] that immunoexpression of galectin-3 was strong in 42% of the CRC galectin-3positive expression cases.

In the present study 50% of all cases showed cytoplasmic and weak nuclear staining which was encountered in moderately differentiated tumors. Cytoplasmic staining with membranous reinforcement was detected in 11.7% of all cases which was encountered in well differentiated tumors. Nuclear and weak cytoplasmic staining was detected in 20% of all cases (seven cases of poorly differentiated tumors and five cases of mucoid carcinoma). Finally, in 18.3% of all cases galectin-3 expression was negative (seven cases of mucoid carcinoma, two cases of signet ring carcinoma and two cases of moderately differentiated tumors) (Table 3) and although no statistical relationship could be reported between the pattern of galectin-3 expression and the histological types (Table 5), these results were in concordance to what was observed by *Arfaoui-Toumi et al.* [21] that immunohistochemical analysis of gal-3 in CRC cases showed that expression of gal-3 was intense and diffuse and almost constantly cytoplasmic with membranous reinforcement in the well differentiated adenocarcinoma. When comparing well differentiated, moderatelydifferentiated and poorly differentiated tumors, they noted a change of gal-3 expression that goes from the membrane and the cytoplasm, to cytoplasm and the nucleus, until it becomes nuclear. Moreover, *Arfaoui-Toumi et al.* [21] observed that in mucinous adenocarcinomas, gal-3 expression decreases meaningfullyin intensity and distribution in the mucinous component of the tumor when compared to the adjacent normal mucosa and to mucinous-free areas

($P < 0.001$). But searching in literature about the relationship between the staining pattern of galectin-3 and the histological grades (Table 6), depth of tumor invasion (Table 7) and modified Dukes' stage (Table 8) didn't reveal similar results.

In the current study no statistically significant relationship could be detected between galectin-3 expression and the age (P value =0.323) (Table 4), this goes in agreement to what was demonstrated by *Endo et al.* [15] and *Tsuboi et al*. [31] that insignificant statistical relationship could be found between galectin-3 expression and age (P value = non-significant & P value = 0.93) respectively.

No statistically significant relationship could be detected between galectin-3 expression and the sex (P value =0.875) (Table 4), this goes in concordance to what was reported by *Endo et al.* [15] and *Tsuboi et al.* [31] that insignificant statistical relationship could be found between galectin-3 expression and sex (P value = non-significant& P value =0.15) respectively.

A statistically significant relationship was detected between galectin-3 expression and the tumor site (P value=0.038) (Table 4), where galectin-3 expression positive cases were predominantly in rectum, considering that the rectum was the predominating site in the current study (17 cases). This was in contrast to what was reported by*Endo et al.* [15] that insignificant statistical relationship could be found between galectin-3 expression and tumor site (P value = non-significant).

In our study a statistically significant relationship was detected betweengalectin-3 expression and the histological type where (89.8%) of conventional adenocarcinoma cases were galectin-3 positive while (63.6%) of mucoid adenocarcinoma cases were galectin-3negative (P value <0.001) (Table 4), this coincides with what was noted by *Endo et al.* [15] (P value = 0.0037).

A statistically significant relationship was detected betweengalectin-3 expression and the histological grade where (100%) of grade I, (93.75%) of grade II and (57.2%) of grade III were galectin-3 expression positive (P value=0.002) (Table 4). This goes in agreement with what was reported by *Tsuboi et al.* [31] and *Dawson et al.* [36] about the correlation between the tumor grade and galectin-3 expression (P value=0.002 & P value=0.0376) respectively. This alsogoes in accordance to what was reported by *Arfaoui-Toumi et al.* [21] that there was progressive decrease of galectin-3 staining in relation to the decreasing degree of tumoral differentiation.

In the current study no statistically significant relationship could be detected in our study between galectin-3 expression and the depth of tumor invasion (P value =0.07) (Table 4), which was in opposite to what was reported by *Endo et al.* [15] *& Tsuboi et al.* [31] that a statistically significant relationship could be found between galectin-3 expression and the depth of tumor invasion (P value =0.01 &P=0.02) respectively.

No statistically significant relationship could be detected between galectin-3 expression and the lymph nodes metastasis in our study (P value =0.833) (Table 4),

which was in accordance to what was reported by *Tsuboi et al.* [31] where in their study there was no statistically significant relationship could be detected between galectin-3 expression and the lymph nodes metastasis(P value = 0.17), however this was in opposite to what reported by *Endo et al.* [15] and *Dawson et al.* [36] that a statistically significant relationship could be found between galectin-3 expression and the lymph nodes metastasis (P value =0.0007 & P value=0.0069) respectively.

In our study no statistically significant relationship could be detected between galectin-3 expression and the distant metastasis (P value =0.633) (Table 4), this was in opposite to what was noted by *Endo et al.* [15] that a statistically significant relationship could be found between galectin-3 expression and the distant metastasis (P value = 0.015).

A statistically significant relationship was detected betweengalectin-3 expression and modified Dukes' stagewhere (98%) of positive galectin-3 expression cases where modified Dukes' stage B&C each was (49%) (P value=0.01789) (Table 4), these figures were in agreement to what was reported by *Endo et al.* [15] regarding the relationship between modified Dukes' stageand galectin-3 expression among their studied cases (P value=0.0004).These figures weren't coinciding with what was reported by *Nagy et al.* [29] that insignificant variation was observed in galectin-3 expression when the Dukes' stages increased from A to C. Moreover, *Nagy et al.* [29] also noted a positive correlation between increasing levels of galectin-3 expression and shorter survival periods, particularly in the case of patients with Dukes' A and B colon tumors.

In spite of the fact that in the current study and other studies examined the immunohistochemical expression of galectin-3 in colorectal cancer reported high level of galectin-3 positivity, however, different sample size, variable grades and pathologic stages enrolled in the studies might explain the contradictory results regarding correlation between galectin-3 expression in CRC and other clinicopathological parameters.

CONCLUSION

The high percentage of galectin-3 immunostaining in colorectal carcinomas cases supports the hypothesis that galectin-3 may act as a prognostic marker to predict poor outcome of patients with colorectal carcinoma. A possible involvement of galectin-3 expression in tumor invasion in patients with colorectal cancer is suggested.Galectin-3 plays a role in the ability of colon cancer cells to metastasize. According to these it is recommended that a scoring system can be applied for galectin-3 expression in CRC cases and could be useful in predicting the outcome in these patients. In order to elucidate the possible prognostic significance of galectin-3 in colorectal carcinoma, it will be necessary to carry out similar studies on a larger sample size and look for correlation between galectin-3 expression and survival rate.It is possible that in the near future, galectin-3 may become an attractive target for the development of new strategies in the diagnosis and treatment of CRC in order to reduce the invasive and metastatic potential of CRC.

Abbreviations:

AJCC : American joint committee on cancer
CRC : Colorectal cancer or colorectal carcinoma
Gal-3 : Galectin-3
TNM : Which referred to the p category of pathological, the T category of tumor, the N category of nodes, the M category of metastasis

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