DOI: 10.7860/JCDR/2021/47792.15092

Original Article

Pathology Section

A Study on the Assessment of Galectin-3 Expression in Colorectal Neoplasm and its Relationship with Tumour Stage, in Tertiary Care Hospital, Kolkata, India

NANDINI BHADURI BHATTACHARYYA[1], ANADI ROY CHOWDHURY[2], SUSMITA MUKHOPADHYAY[3], SNEHA[4]

ABSTRACT

Introduction: Galectin-3 is a β-galactoside-binding lectin found in a considerable number of normal tissues and malignant neoplasms. It was found to be expressed in few thyroid tumours particularly follicular and papillary tumours. Lectins were found to be released in circulation and increased concentration was noted in colorectal cancers especially in metastatic colonic adenocarcinoma. Different types of galectins are expressed in normal colonic and rectal epithelium. Some types do increases in inflammation and cancers of these areas. It was seen that galectin-3 increases in colorectal tumourigenesis and it bears an important role in cancer progression and metastasis. Galectin-3 seems to have an important role in colorectal cancer. Some studies proved that galectin inhibitors could reduce tumour progression and metastasis and it may be a therapeutic target in metastatic colorectal adenocarcinoma.

Aim: To evaluate colon cancer specimens received for biopsy, for galectin-3 expression and its relation with tumour stage, lymphovascular space invasion and tumour differentiation.

Materials and Methods: The study was a cross-sectional observational study conducted in the Department of Pathology of RG Kar Medical College and Hospital from November 2018 to November 2019. It was an immunohistochemistry based assay performed to test the expression levels of galectin-3 in cancer tissues of 62 colorectal neoplasms with the help of galectin-3 primary antibody (mouse monoclonal antibody- clone 9C4). Statistical analysis was done using Statistical Package of Social Sciences (SPSS) version 19.0.

Results: Out of 62 cases, 60 cases were colorectal adenocarcinoma and 2 cases were adenoma with age group between 40-75 years. Total 46 cases had cancer in caecum and ascending colon and rest were in recto-sigmoid colon. A 36/60 cases (60%) of cancer tissues were positive for galectin-3 expression. Strong association of lymphovascular space invasion (p=0.046) and depth of tumour (p=0.0078) with galectin positivity in colon carcinoma was noted.

Conclusion: Evaluation of galectin-3 expression is helpful in the assessment of tumour staging and prognosis in colorectal cancer patients. It may have a therapeutic implication in the management of colon cancer in future.

Keywords: Colon cancer, Galactose binding, Immunohistochemistry, Lectin

INTRODUCTION

Galectin-3 is an endogenous carbohydrate binding protein that is implicated in cell growth, differentiation, adhesion, malignant transformation and apoptosis. It is predominantly located in the cytoplasm. Galectin-3 seems to have an important role in colorectal cancer. Dabbs DJ examined the expression of galectin-1 and 3 in a series of few thyroid tumours and reported expression of these lectins in papillary and follicular carcinomas, but not in adenomas, nodular goitre, or normal thyroid tissue. Based on these studies, they concluded that the galectins could be useful in the distinction of benign and malignant thyroid tumours [1].

There is strong in-vitro and in-vivo evidence that tumourigenesis and metastasis can be reduced by galectin inhibitors. Galectin-3-null mice are relatively healthy, indicating that inhibition of galectin-3-mediated actions may present a viable and relatively safe therapeutic approach for cancer treatment [2].

Galectin-3 is also released into the circulation. Concentrations of circulating galectin-3 in the bloodstream of colorectal cancer patients can be increased up to five fold [3]. Moreover, patients with metastasis have higher levels of circulating galectin-3 than those with localised tumours [4]. The beta subunit of haptoglobin co-precipitates with galectin-3 from the serum of patients with colorectal cancer [4]. Recent studies have suggested that the increased circulation of galectin-3 in the bloodstream of cancer patients can be an important promoter of cancer cell metastasis [5,6].

In the normal human colon and rectum, four galectins, galectin(gal)-1, 3, 4 and 8, are expressed [7-10]. Galectin-1 is expressed weakly in normal colonic epithelium but its expression is increased in inflammation and cancer. The results on gal-1 and gal-3 clearly show that galectins are involved in the malignant progression of colon cancer and their migration properties [11].

Differential expression of galectin expression is very much common in cancers of the human gastrointestinal tract. Accumulating evidences support an active role of these endogenous carbohydrates binding protein in the regulation of colorectal cancer development, progression and distant metastasis [12].

Galectin-3 expression is greater in advanced cancer [13] and metastases express higher levels of galectin-3 than the primary tumours from which they arise [14]. There is a general change in galectin-3 sub-cellular localisation from nucleus to the cytoplasm in colorectal cancer during progression from colorectal adenoma to carcinoma [8,15]. As cytoplasmic galectin-3 is known to be an apoptosis inhibitor, it is very likely that this change in localisation may contribute to cancer cell survival.

Galectin-4 is expressed in the human intestinal and colonic mucosa and its expression is generally lower in cancer than in normal mucosa [9,16,17].

Galectin-8 is expressed widely in the gastrointestinal tract. Low basal levels of galectin-8 are observed in the human intestine [18,19]. Hence, the present study aimed to evaluate the colon cancer

specimens for galectin-3 expression and its relation with tumour stage, lymphovascular space invasion and tumour differentiation.

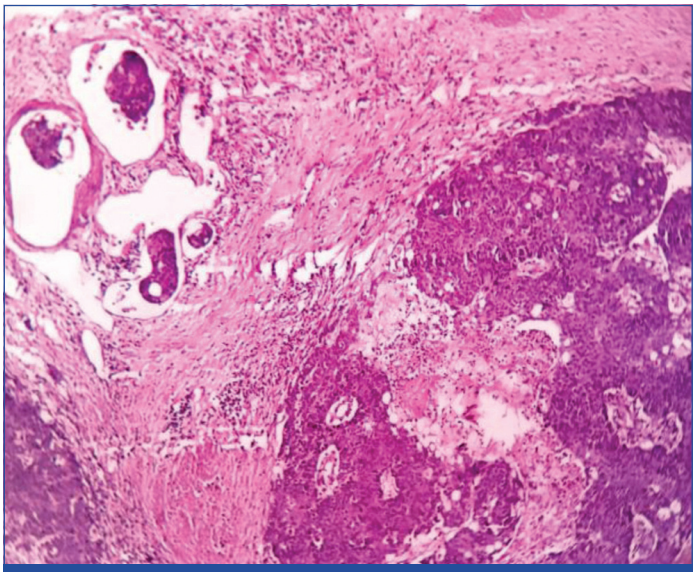
MATERIALS AND METHODS

The present study was an observational cross-sectional study carried out in the Department of Pathology, RG Kar Medical College, Kolkata, India from November 2018 to November 2019. Total 62 colorectal specimen with growth were collected during this period. Out of 62 cases, 40 cases were from male patients and 22 cases were from female patients. Institutional Ethics Committee permission (IEC No RKC/47) was taken.

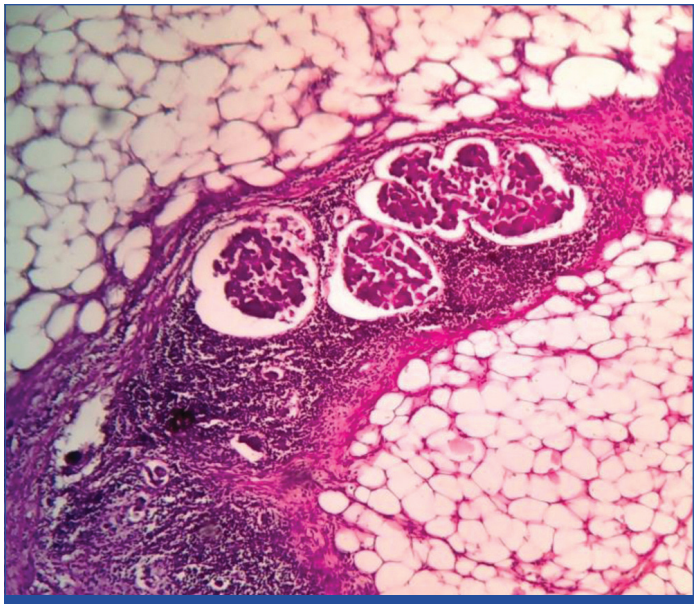
Study Procedure

Surgically resected colorectal cancer tissues were fixed in 10% formalin and embedded in paraffin for routine pathological diagnosis. Paraffin blocks containing representative cancer tissue were selected and used for immunohistochemical study to see galectin 3 expression. The final result was achieved by multiplying the scores from the 2 categories: 0 to 3 points is negative expression; 4 to 6 points weakly positive expression (+), 7 to 9 points, fairly strong positive expression (++), 9 to 12 points, strong positive expression (+++) [13].

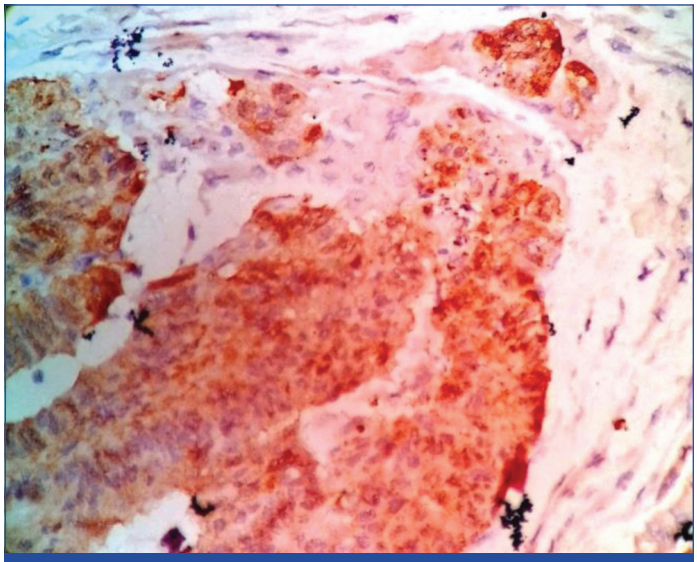
Tumour staging was done from assessment of depth of tumour penetration, lymphovascular invasion and lymph node metastasis. The IHC-2 sections of 2-3 µm thickness were prepared, one for H&E staining and one for IHC with galectin-3 primary antibody (mouse monoclonal antibody- clone number 9C4). The samples were evaluated by cell staining with the following scores: brown- **3 points**; pale brown**- 2 points**; light brown- **1 point**; and no colouration- **0 points**. The quantity of stained cells was evaluated with the following scores: quantity of stained cells in one visual field of >75%- **4 points**; 51% to 75%- **3 points;** 11% to 50%- **2 points**; less than 10%- **1 point**, and negative- **0 points** [13]. [Table/Fig-1a]: Poorly differentiated colorectal carcinoma with Lymphovascular Space Invasion (LVSI) in surrounding area (100X magnification, H&E). [Table/Fig-1b]: Tumour deposit in the surrounding adipose tissue with LVSI. (100X magnification, H&E). [Table/Fig-1c]: strong positive galectin stain of 3×4=12 score (IHC) and [Table/Fig-1d]: weakly positive galectin stain of 2×2=4 score (IHC) in this study.



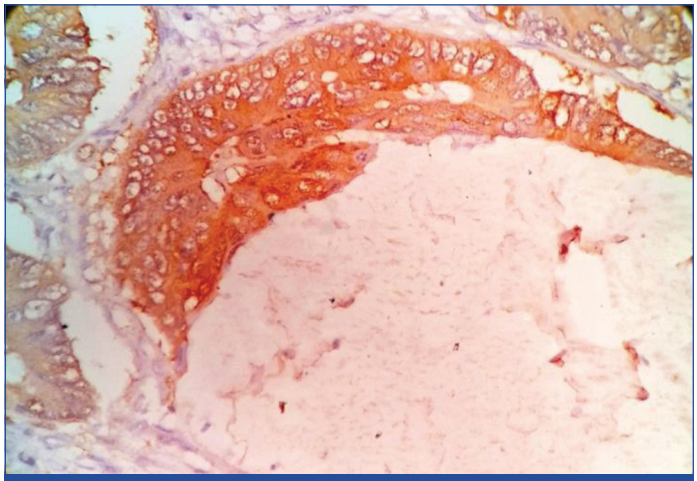
[Table/Fig-1a]: Poorly differentiated colorectal carcinoma with LVSI in surrounding area. (100X magnification, H&E).
LVSI: Lymphovascular space invasion



[Table/Fig-1b]: Tumour deposit in the surrounding adipose tissue with LVSI. (100X magnification, H&E).
LVSI: Lymphovascular space invasion



[Table/Fig-1c]: Strong positive immunohistochemical expression of galectin-3 (Score 12) in colorectal carcinoma (400X magnification, Immunohistochemistry with galectin-3 clone number is 9C4).



[Table/Fig-1d]: Weakly Positive Immunohistochemical expression of galectin-3 (Score 4) in colorectal carcinoma (400X magnification, immunohistochemistry with galectin-3 clone number is 9C4).

STATISTICAL ANALYSIS

Statistical analysis was done using 2×2 contingency table from each category with the help of SPSS version 19.0 and Chi-square test was done to assess level of significance.

RESULTS

In this study the expression of galectin-3 in 62 tissue specimens were assessed, which included 2 adenomas (one rectal villous adenoma and one was traditional serrated adenoma in ascending colon) and

60 cases of colon carcinomas of known Tumour Node Metastasis (TNM) stage. Therefore, the result analysis was done on 60 cases only, excluding the adenomas. The average age of 62 patients in this study was between 40 years to 75 years. There were 40 males and 22 females. A total of 46 cases had tumour in ascending colon and caecum and 16 had tumour in recto-sigmoid region. Total 36 out of 60 (60%) of the tumours showed galectin-3 expression. The various parameters studied for galectin-3 expression are: 1) Tumour stage; 2) LVSI; and 3) Tumour differentiation.

Clinicopathologic characteristics of all the cases are shown in [Table/Fig-2]. There is strong association (p-value <0.05) of Lymphovascular space invasion and galectin positivity in colon carcinoma which is depicted in [Table/Fig-3]. Strong association was also noted in cases of depth of tumour and galectin positivity (p-value=0.014 i.e., p<0.05) noted in [Table/Fig-4].

Tumour site	Number of cases
Caecum and ascending colon	46
Recto-sigmoid	16

[Table/Fig-2]: Clinicopathologic characteristics of all 62 cases (including the 2 adenomas).

LVSI	Number	Galectin positive	Galectin negative	p-value
Present	39	27	12	0.046
Absent	21	9	12	

[Table/Fig-3]: Association between galectin-3 expression and lymphovascular space invasion. N=60 (only the cancer cases).
Chi-square test; LVSI: Lymphovascular space invasion

Tumour stage	Number	Galectin positive	Galectin negative	p-value
Stage T2	17	06	11	0.014
Stage T3&T4	43	30	13	

[Table/Fig-4]: Association between galectin-3 expression and depth of tumour invasion. n=60 (only the cancer cases).
Chi-square test

There was also increased negativity of galectin in relation to tumour differentiation in colorectal carcinoma shown in [Table/Fig-5]. Poorly differentiated carcinoma show 13/19 (68.4%) negative expression compared to well differentiated carcinoma where only 5 out of 13 cases (38.4%) showed negative expression.

Differentiation of tumour	Number	Galectin positive (n=36)	Galectin negative (n=24)	p-value
Well-differentiated	13	8	5	0.0054
Moderately differentiated	28	22	6	
Poorly differentiated	19	6	13	

[Table/Fig-5]: Association between Galectin-3 expression and differentiation of tumour. n=60 (only the cancer cases)
Chi-square test

DISCUSSION

Galectin-3 is an endogenous carbohydrate binding protein implicated in cell growth, differentiation, adhesion, malignant transformation and apoptosis. Being predominantly located in the cytoplasm, it seems to have an important role in colorectal carcinogenesis.

In the present study, patients were in stage 2 in 17 cases and in stage 3 and stage 4 combined in 43 cases. 6 cases of stage 2 tumour, were galectin-3 positive and 30 cases in stages 3 and 4 were galectin-3 positive. The p-value was 0.014 and significant (p<0.05).

A total of 39 samples were LVSI positive and 27 of them were galectin-3 positive. Twenty one cases were negative for LVSI. Out of LVSI negative cases 9 were galectin-3 positive. The p-value was calculated to be 0.046, result is significant at p<0.05.

Similar study was done by Schoeppner HL et al., on galectin-3 expression in 153 tissue specimens. They found galectin-3 expression was significantly higher in high grade dysplasia and early invasive cancers compared with the adenomatous tissue. A linear relationship noted between galectin-3 expression in invasive cancers with advancing stage (p=0.008). Decreased long term patient survival (p=0.021) correlated with enhanced expression. Higher level of galectin-3 expression noted in metastasis compared with the primary cancers (p<0.005) [14].

A similar study using immunohistochemistry assay was used to test the expression status of galectin-3 in cancer tissues of 61 colorectal cancer (61 cases) and in normal intestinal mucosa adjacent to the cancer (23 cases). Clinicopathological features, such as age, sex, pathological type, lymphatic metastasis, and prognosis were also analysed with the galectin-3 level. Rate of positivity of galectin-3 in cancer tissues was significantly higher than that of normal epithelium adjacent to cancer 62.5% (38/61) versus 13.0% (3/23) (p<0.05) respectively. Positive correlation was noted between the protein expression of galectin-3 and the tumour size (p<0.05), tumour differentiation (p<0.05) and duke's stage (p<0.05) [20].

Another similar study conducted in Kyushu University, Fukuoka, Japan on 121 colorectal cancer patients, showed positive expression in 65% patients. The incidence of lymph node metastasis was significantly high in galectin-3 positive cases with significance level p<0.05 (p=0.0007). Deeper invasion into wall and lymphatic permeation was higher in positive cases (p=0.01 and p=0.041, respectively). Larger tumour size had higher expression (p=0.016) [21].

Another study conducted in Department of General Surgical Science of Gunma University Graduate School of Medicine, Maebashi, Japan on 108 patients with colorectal cancer was investigated using immunohistochemical analysis. Galectin-3 expression at the surface of the tumour was correlated with the depth of invasion (p=0.02) and blood vessel invasion (p<0.01) in this study [22].

A study from a chinese article showed that galectin 3 was expressed in 158 cases including 30 normal mucosa, 25 adenomas, 65 carcinomas and 38 metastatic tumour specimens. Normal tissues showed different expression than adenomas (p<0.001). Poorly differentiated and colorectal cancer with metastasis showed higher expression than well and moderately differentiated tumours (p=0.03 and p<0.001, respectively). Invasive tumours had higher expression than non invasive tumours (p<0.001) [23].

Limitation(s)

Not all parameters were satisfactorily described in the present study because of low sample size. It can be made more statistically significant if a large sample size would be taken.

CONCLUSION(S)

Immunohistochemical detection of elevated expression of galectin-3 is a potent prognostic marker in colorectal cancer, but its biological function is still to be explored. Elucidation of this function may contribute to our search for a new therapeutic regime against cancer progression and metastasis in future. The higher expression of galectin-3 may contribute as a metastasis predictor for colorectal carcinoma. This study is restricted in this regard due to low sample volume. Further studies are needed.

Acknowledgement

Authors would like to thank Prof. (Dr.) Tushar Kanti Das, Head of the Department, Department of Pathology, and all technical staff of the department.

REFERENCES

[1] Dabbs DJ. Immunohistology of Endocrine Tumours. In: Diagnostic immunohistochemistry: Theranostic and genomic applications. 5th ed. Philadelphia: Elsevier; 2019. Pp. 359.
[2] Colnot C, Ripoche MA, Milon G, Montagutelli X, Crocker PR, Poirier F. Maintenance of granulocyte numbers during acute peritonitis is defective ingalectin-3-null mutant mice. Immunology. 1998;94:290-96.

[3] Iurisci I, Tinari N, Natoli C, Angelucci D, Cianchetti E, Iacobelli S. Concentrations of galectin-3 in the sera of normal controls and cancer patients. Clin Cancer Res. 2000;6(4):1389-93.
[4] Bresalier RS, Byrd JC, Tessler D, Lebel J, Koomen J, Hawke D, et al. A circulating ligand for galectin-3 is a haptoglobin-related glycoprotein elevated in individuals with colon cancer. Gastroenterology. 2004;127(3):741-48. Doi: 10.1053/j.gastro.2004.06.016.
[5] Zhao Q, Guo X, Nash GB, Stone PC, Hilkens J, Rhodes JM, et al. Circulating galectin-3 promotes metastasis by modifying MUC1 localization on cancer cell surface. Cancer Res. 2009;69(17):6799-806. Doi: 10.1158/0008-5472.CAN-09-1096.
[6] Yu LG. Circulating galectin-3 in the bloodstream: An emerging promoter of cancer metastasis. World J Gastrointest Oncol. 2010;2(4):177-80. Doi: 10.4251/wjgo.v2.i4.177.
[7] Demetter P, Nagy N, Martin B, Mathieu A, Dumont P, Decaestecker C, et al. The galectin family and digestive disease. J Pathol. 2008;215(1):01-12.
[8] Sanjuan X, Fernandez PL, Castells A, Castronovo V, van den Brule F, Liu FT, et al. Differential expression of galectin 3 and galectin 1 in colorectal cancer progression. Gastroenterology. 1997;113(6):1906-15.
[9] Huflejt ME, Leffler H. Galectin-4 in normal tissues and cancer. Glycoconj J. 2004;20(4):247-55.
[10] Nagy N, Bronckart Y, Camby I, Legendre H, Lahm H, Kaltner H, et al. Galectin-8 expression decreases in cancer compared with normal and dysplastic human colon tissue and acts significantly on human colon cancer cell migration as a suppressor. Gut. 2002;50(3):392-401.
[11] Hittelet A, Legendre H, Nagy N, Bronckart Y, Pector JC, Salmon I, et al. Upregulation of galectins-1 and -3 in human colon cancer and their role in regulating cell migration. Int J Cancer. 2003;103(3):370-79.
[12] Barrow H, Rhodes JM, Yu LG. The role of galectins in colorectal cancer progression. Int J Cancer. 2011;129(1):01-08.
[13] Povegliano LZ, Oshima CTF, de Oliveira Lima F, Scherholz PLA, Forones NM. Immunoexpression of Galectin-3 in colorectal cancer and its relationship with survival. J Gastrointest Cancer. 2011;42(4):217-21.
[14] Schoeppner HL, Raz A, Ho SB, Bresalier RS. Expression of an endogenous galactose-binding lectin correlates with neoplastic progression in the colon. Cancer. 1995;75(12):2818-26.
[15] Andre S, Kojima S, Yamazaki N, Fink C, Kaltner H, Kayser K, et al. Galectins-1 and -3 and their ligands in tumour biology. Non-uniform properties in cell-surface presentation and modulation of adhesion to matrix glycoproteins for various tumour cell lines, in biodistribution of free and liposome bound galectins and in their expression by breast and colorectal carcinomas with/without metastatic propensity. J Cancer Res Clin Oncol. 1999;125(8-9):461-74.
[16] Lahm H, Andre S, Hoeflich A, Fischer JR, Sordat B, Kaltner H, et al. Comprehensive galectin fingerprinting in a panel of 61 human tumour cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. J Cancer Res Clin Oncol. 2001;127(6):375-86.
[17] Nagy N, Legendre H, Engels O, Andre S, Kaltner H, Wasano K, et al. Refined prognostic evaluation in coloncarcinoma using immunohistochemical galectin fingerprinting. Cancer. 2003;97(8):1849-58.
[18] Gopalkrishnan RV, Roberts T, Tuli S, Kang D, Christiansen KA, Fisher PB. Molecular characterization of prostate carcinoma tumour antigen-1, PCTA-1, a humanGalectin-8 related gene. Oncogene. 2000;19(38):4405-16.
[19] Su ZZ, Lin J, Shen R, Fisher PE, Goldstein NI, Fisher PB. Surface-epitope maskingand expression cloning identifies the human prostate carcinoma tumour antigen gene PCTA-1 a member of the galectin gene family. Proc Natl Acad Sci USA. 1996;93(14):7252-57.
[20] Tao L, Jin L, Dechun L, Hongqiang Y, Changhua K, Guijun L. Galectin-3 expression in colorectal cancer and its correlation with clinical pathological characteristics and prognosis. Open Med (Wars). 2017;12:226-30. Doi: 10.1515/med-2017-0032.
[21] Endo K, Kohnoe S, Tsujita E, Watanabe A, Nakashima H, Baba H, et al. Galectin-3 expression is a potent prognostic marker in colorectal cancer. Anticancer Res. 2005;25(4):3117-21.
[22] Tsuboi K, Shimura T, Masuda N, Munenori IDE, Tsutsumi S, Yamaguchi S, et al. Galectin-3 expression in colorectal cancer. Anticancer Research. 2007;27:2289-96.
[23] Zhang N, Ding YQ, Liang I. Association of galectin-3 expression with biological behaviors of human colorectal carcinoma. Nan Fang Yi Ke Da Xue Bao. 2006;26:1685-89.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, RG Kar Medical College, Kolkata, West Bengal, India.
2. Professor, Department of Pathology, RG Kar Medical College, Kolkata, West Bengal, India.
3. Assistant Professor, Department of Pathology, RG Kar Medical College, Kolkata, West Bengal, India.
4. Junior Resident, Department of Pathology, RG Kar Medical College, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Anadi Roy Chowdhury,
Kailash 502, Godrej Prakriti, 187f/1 BT Road, Sukhchar, West Bengal, India.
E-mail: dr-anadi@hotmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 17, 2020
- Manual Googling: Feb 13, 2021
- iThenticate Software: Mar 15, 2021 (19%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Nov 15, 2020**
Date of Peer Review: **Dec 31, 2020**
Date of Acceptance: **Feb 17, 2021**
Date of Publishing: **Jul 01, 2021**