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Original Research Article

Immunohistochemical expression of galectin-3 in carcinoma of uterine cervix

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ABSTRACT

Background: Cervical cancer is one of the most common cancer among women worldwide, second to breast cancer. It's difficult to diagnose in the early stage due to subtle and non-specific symptoms. Galectin-3 is increasingly being recognized as an important regulator of a broad range of cancer cell activities including cervical cancer. Its expression depends on the tumor grade which signifies its role in progression of the disease and prognostic significance.
Method: The present study was conducted in department of pathology, Pt. B. D. Sharma PGIMS, Rohtak on 60 cases of histo-pathologically diagnosed squamous cell carcinoma of uterine cervix. Immunohistochemical (ICH) expression of marker 'Galectin-3'was assessed in all biopsy specimens.
Results: Galectin-3 expression was 1+ in 83.3% cases and 2+ in 16.6% cases of grade 1 cervix carcinoma. In grade 2 carcinomas 55.3% showed 1+, 42.1% showed 2+ and 2.6 % cases showed 0 galectin -3 expression. Further, 70% of grade III cases showed a score of 2+, and 10 % showed 1+ expression and 20 % didn't show any galectin 3 positivity. A statistically significant association of galectin-3 expression with histological grade was observed.
Conclusions: IHC expression of galectin-3 can be used as an important marker for grading of cervical carcinoma, therefore the present study was done to evaluate the IHC expression of galectin 3 in cervical cancer and correlate it with histologic tumour grade.

Keywords: IHC, Galectin 3, Tumor grade

INTRODUCTION

Cervical cancer is one of the most common cancer among women worldwide, second to breast cancer.[1] Its difficult to diagnose in the early stage due to subtle and nonspecific symptoms. The main cause of preneoplastic and neoplastic lesions of cervix is the persistent HPV infection (HPV 16 and 18 mainly) along with other etiological factors including smoking, reproductive factors, nutritional factors (like lack of folic acid) and genetic factors etc.[2] Papanicolaou (Pap) smear has significantly contributed to the decreasing incidence of cervical cancer but due to its low sensitivity there is a need of more accurate biologic marker.[3] Galectin-3 is increasingly being recognized as an important regulator of a broad range of cancer cell activities. The level of expression of galectin-3 depends on the tumor type analyzed.[4] Its expression has been studied in various tumors including its influence on vascular endothelial growth factor C expression and enhancement of cervical cancer cell invasiveness.[5] There are very few studies on the role of galectin -3 in cervical cancer which show variable intensities of expression with increasing grade of tumor. Therefore, the present study was done to evaluate the IHC expression of galectin 3 in different histological tumour grades of cervical cancer and also to determine its role in prognosis and development of new treatment modalities targeting galectin-3.

METHODS

The present study was conducted in department of pathology, Pt. B D Sharma PGIMS, Rohtak. The current study was a prospective observational study and the data was collected over a period of one year between July 2019 to June 2020. Sixty cases of histo-pathologically proven squamous cell carcinoma of uterine cervix biopsy were included in the study and patients having any treatment including chemotherapy/ radiotherapy were excluded from the study.

All the biopsy specimens were subjected to careful and detailed gross examination, fixed in 10% buffered formalin, routinely processed and embedded in paraffin. The sections prepared were stained with (H and E) haematoxylin and eosin stain and further IHC profile was assessed by subjecting one section from the representative block to galectin-3 immuno-stain.

IHC and H and E staining were carried out according to the standard procedure.[6,7] This study is ethically approved.

Interpretation

The interpretation of intensity and extent of galectin-3 cytoplasmic staining were based on Lee et al study.[2]

The staining intensity was graded on a scale of 0 to 2+ as follows: 0: weak intensity, 1+: weak to moderate intensity, 2+: moderate to strong intensity, the extent of staining was scored as follows: 0:<10% stained, 1+: 10 - 50% stained, 2+: >50% stained.

The distribution and intensity of cell staining were assessed with observation of entire tumor areas and at least 10 high-power field (HPF) areas. The percentage of cells expressing galectin-3 were estimated by dividing the number of positively stained tumor cells by the total number of tumor cells per HPF. Cervical tissues having any other disease except carcinoma cervix, obtained from hysterectomy specimens served as a positive control of galectin-3. Negative control sections were made by exclusion of the primary galectin-3 antibody.

Statistical analysis

The collected data was categorized and coded as appropriate. The data was then compiled, tabulated and analysed using SPSS 24 software. The statistical tests including percentages, proportions, chi-square test and unpaired t test were applied wherever applicable. P<0.05 was considered statistically significant.

Biomedical waste disposal

All the biomedical waste generated during the study was discarded as per the biomedical waste management and handling, rules 2018.[8]

RESULT

The study comprised of sixty cases of squamous cell carcinoma of uterine cervix. Histopathological diagnosis was established on routine H and E-stained section (Figure 1).

IHC profile of the tumor was assessed by subjecting one section each from a representative block of tumor biopsy to galectin-3. Galectin-3 expression was then correlated with the various clinicopathological parameters such as the age of the patient, the histological grade and size of biopsy. Chi square test was applied to assess the association and a value of $p<0.05$ was taken as significant.

The cases selected for the study was in the age group of 21 to 80 years with the class interval of 10 years and mean age of 53.18 years. Maximum cases (n=19, 31.6%) were seen in the age group of 41-50 and 51-60 years, followed by cases in the age group of 61-70 years (n=9, 15%).

Cases showed variable symptoms at onset. 44 out of 60 cases were having bleeding per vaginum, 12 cases were having discharge per vaginum, 2 cases were having post coital bleeding and 2 cases were having pain abdomen.

Distribution of cases was also done according to biopsy size. Maximum cases (n=51, 85%) were having biopsy size $\geq$1 cm. Only 9 cases (15%) were of size <1 cm.12 out of 60 cases were of WDSCC, 38 cases were of moderately differentiated squamous cell carcinoma (MDSCC), and 10 cases were of poorly differentiated squamous cell carcinoma (PDSCC). Out of 60 cases, maximum number of cases (n=32, 53.3%) showed galectin 3 expression of 1+, followed by Galectin 3 expression of 2+ (41.7%).

In the age group of > 50 years (n=33), 2 cases revealed 0+ galectin-3 expression, 21 cases revealed 1+ expression and 10 cases revealed 2+ expression, respectively. In patients less than and equal to 50 years (n=27), 1 case revealed 0+ galectin-3 expression, 11 cases revealed 1+ expression, and 15 cases revealed 2+ galectin-3 expression. The difference in expression of galectin-3 in different age groups was statistically non-significant (Pearson's correlation coefficient=14.715 and p=0.143).

On comparing the expression of galectin -3 in different grades of cancer we found that out of 60 cases, 12 cases were of WDSCC type of which 10 revealed 1+ galectin-3 expression and 2 cases showed 2+ galectin-3 expression. 38 cases were of MDSCC type of which 1 case revealed 0+ galectin 3 expression, 21 cases showed 1+ galectin- 3 expression and 16 cases showed 2+ galectin 3 expression. 10 cases were of PDSCC type of which 2 revealed 0+ galectin 3 expression, 1 case showed 1+ galectin 3 expression and 7 cases showed 2+ galectin 3 expression. The difference in expression of galectin-3 in various

tumour types was statistically significant (Chi square=14.827, p=0.005). Galectin-3 expression in PDSCC was significantly increased compared to that in WDSCC and MDSCC (p=0.002 and p=0.013, respectively). But there were no significant differences in the level of expression between WDSCC and MDSCC (p=0.211). Table number 1 shows relationship of galectin-3 expression with tumor grades and Figure 2 and 3 shows level of galectin-3 expression (1+ and 2+).

Out of 60 cases, 9 cases were of biopsy size <1 cm of which 3 cases revealed 0+ galectin-3 expression and 5 cases showed 1+ galectin-3 expression and 1 case showed 2+ galectin -3 expression. 51 were of biopsy size ≥1 cm of which 28 cases showed 1+ galectin- 3 expression and 23 cases showed 2+ galectin 3 expression. The difference in expression of galectin-3 in various biopsy size was statistically significant (Chi square=19.2, p=0.001). Table 2 shows correlation of galectin-3 expression with different clinicopathological parameters.

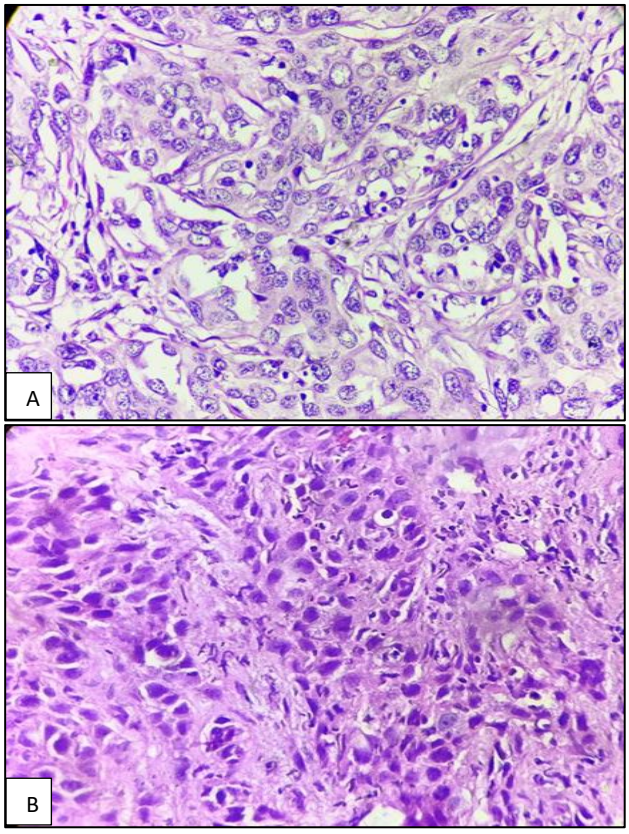

Figure 1 (A and B): Hematoxylin and eosin (H and E) (400x) stain showing MDSCC and PDSCC.

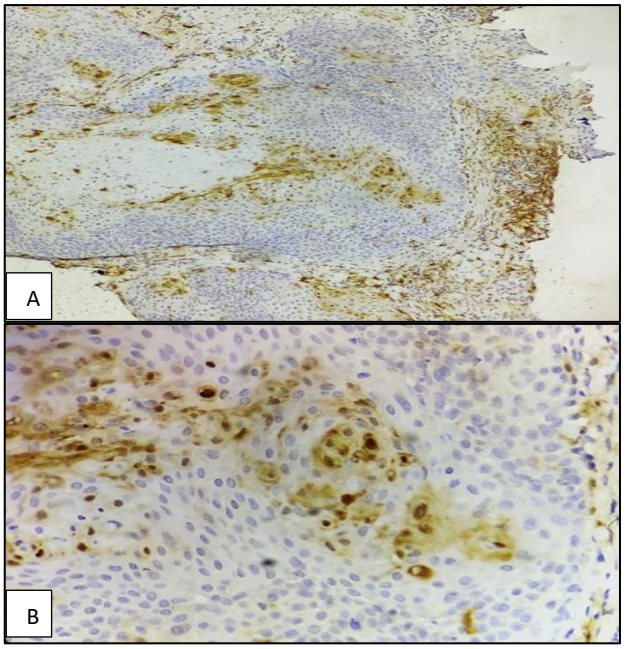

Figure 2 (A and B): IHC stain showing galectin-3 expression=1+ 200X and 400X.

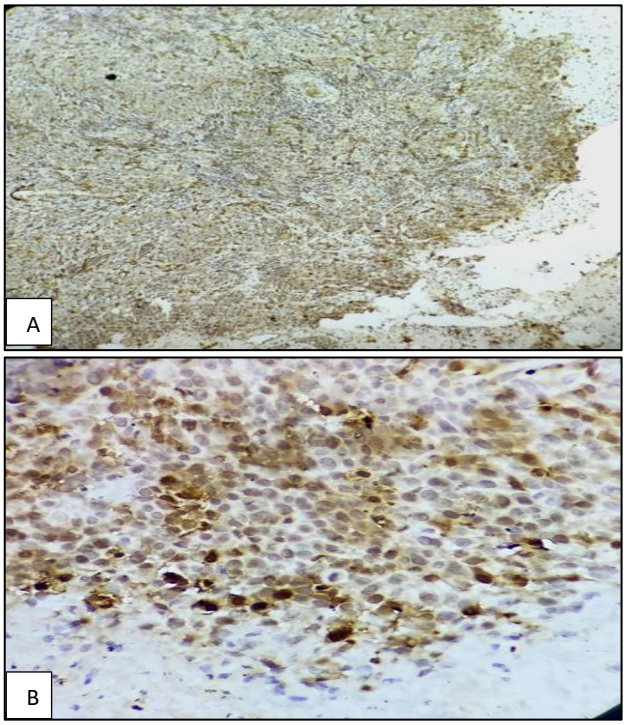

Figure 3 (A and B): IHC stain showing galectin-3 expression=2+ 200X and 400X.

Table 1: Relationship of galectin-3 with tumor grade, (n=60).

Tumour grade	Galectin-3 expression, n (%)				Chi square and p
	0	1+	2+	Total no. of cases	
WDSCC[a] (grade 1), (n=12)	0 (0)	10 (83.3)	2 (16.6)	12	14.827, 0.005
MDSCC[b] (grade 2), (n=38)	1 (2.6)	21 (55.3)	16 (42.1)	38	
PDSCC[c] (grade 3), (n=10)	2 (20)	1 (10)	7 (70)	10	

a-b (p=0.211), b-c (p=0.013), a-c (0.002).

Table 2: Correlation of galectin-3 expression with different clinicopathological parameters.

Clinicopathological parameters		No. of cases	Galectin -3 expression			P value
			0+	1+	2+	
Age (years)	≤50	27	1	11	15	0.186
	>50	33	2	21	10	
Biopsy size (cm)	<1	9	3	5	1	0.001
	≥1	51	0	28	23	
Histologic type	WDSCC	12	0	10	2	0.005
	MDSCC	38	1	21	16	
	PDSCC	10	2	1	7	

DISCUSSION

Cervical cancer is one of the most common gynecologic cancers in women worldwide, second to breast cancer, accounting for around 13% of both total cancer cases and total cancer deaths in women, of which almost 80% are in the developing countries.[1] The main cause of cervical cancer is persistent human papilloma viral (HPV) infection. HPV inactivates the pRb tumour suppressor protein; thus, p16 expression, which is controlled by a negative feedback mechanism, gets increased relatively.[2]

Galectin-3 a member of the lectin family, contributes to oncogenesis, angiogenesis, cancer progression and metastasis through regulation of cell-cell and cell-matrix interactions.[4] Studies of pancreatic, gastric, thyroid, head and neck squamous cell, and renal cell carcinomas found glaectin-3 to be upregulated in these tissues while breast, ovarian and uterine adenocarcinomas showed decreased expression. Although these contradictory results cannot be completely explained, the levels of galectin-3 expression depend on the organ or tissue, suggesting that tumor or tissue-specific factors may modulate the galectin-3 expression; moreover, the heterogeneity of tumor cells, composed of different clones, might be of importance.[9]

In some tumors, a direct relationship was shown between galectin-3 levels and the stage of tumor progression. A wide variation in the expression of galectin-3 was observed, including staining and interpretation. Most studies including present study considered cytoplasmic reactivity of galectin-3 while many others did not mention the staining pattern. Kim et al considered both nuclear and cytoplasmic reactivity.[10]

Present study calculated immunoreactivity score (scored 0 to 2+) which was similar to lee et al. Punt et al, Li et al and Ma et al calculated an immunoreactive score by adding the intensity and extent.[2,11-13]

This study included 60 cases of cervix carcinoma. The incidence rate of cervix cancer increases significantly with age and reaches its peak in the age of menopause and then gradually decreases or remains constant. The mean age of cases was 53.18 (range: 21-80) years, which was comparable to other studies. Maximum cases (n=19, 31.6%) were seen in the age group of 41-50 years and 51-60 years. The mean age was found similar to Kumar et al study. The age range was similar to punt et al and Kumar et al study.[11,14] Other studies did not mentioned mean age and age range. The difference in expression of galectin-3 in different age groups was statistically non-significant (p=0.186), which was in concordance with other studies.

This study observed maximum cases (n=51, 85%) to be of biopsy size more than or equal to 1 cm and minimum cases (n=9, 15%) of size less than 1 cm. The difference of galectin 3 expression was statistically significant (chi – square value 19.2, p=0.001). In other studies biopsy size was not considered for galectin 3 expression evaluation.

In the current study, maximum number of cases (n=38, 63.3%) were of grade II (MDSCC), followed by grade I, WDSCC (n=12, 20%) and grade III, PDSCC (n=10, 16.7%). Galectin-3 expression was 1+ in 83.3% cases and 2+ in 16.6% cases of grade 1 cervix carcinoma. In grade 2 carcinomas 55.3% showed 1+, 42.1% showed 2+ and 2.6 % cases showed 0 Galectin 3 expression. Further, 70% of grade III cases showed a score of 2+, and 10 % showed 1+ expression and 20 % showed 0 Galectin 3 expression. A statistically significant association of galectin-3 expression with histological grade was observed, which was in concordance with other studies. Tumor grade reflects the potential aggressiveness of the cervix cancer and is a strong prognostic factor.

Kim et al, Li et al, Ma et al and Kumar et al observed that there was progressive increase in expression of galectin-3 from LSIL to HSIL, with no difference in HSIL and SCC cases.[10,12-14] A statistically significant association of galectin-3 with histological diagnosis was noted ($p<0.05$). The findings of these studies are in concordance with the present study in which galectin 3 expression was higher in PDSCC (7/10 p=0.005).

Lee et al demonstrated that galectin-3 expression gradually decreased with histopathologic grades from LSIL to ISCC. Punt et al study results suggest that strong galectin-3 expression is correlated with less tumor growth, while weak galectin-3 may be correlated with increased tumor growth.[3,11]

Present study demonstrated that there is significant correlation between galectin-3 expression and histological grades of cervical cancer (p=0.005) and

biopsy size (p=0.001), however there is no significant correlation with age of patients (p=0.186). Present study also demonstrated that galectin-3 expression in PDSCC was significantly increased compared to that in WDSCC and MDSCC (p=0.002 and p=0.013, respectively). But there was no significant difference in the level of expression between WDSCC and MDSCC (p=0.211). This signifies its role in progression of the disease and its prognostic significance. Thus, IHC expression of galectin-3 can be used as an important marker for grading of cervical carcinoma and further in determining prognosis and development of new treatment modalities targeting galectin-3.

Limitations

The sample size selected for this study was less. Further studies, including more number of samples will likely result in the development of novel approaches for determining prognosis and development of new treatment modalities targeting galectin-3.

CONCLUSION

The current study found that there is significant increase in galectin 3 expression in poorly differentiated squamous cell carcinoma compared to WDSCC and MDSCC. Hence galectin-3 may be considered as a useful marker in prognosis and grading of squamous cell carcinoma of uterine cervix.

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REFERENCES

1. World Health Organization. Cervix cancer: prevention and control. Available at: https://www.who.int/cancer/cervical-cancer. Accessed on 11 September 2019.
2. Lee JW, Song SY, Choi JJ, Choi CH, Kim TJ, Kim J et al. Decreased galectin-3 expression during the progression of cervical neoplasia. J Cancer Res Clin Oncol. 2006;132:241-7.
3. World Health Organization. Cervix cancer: prevention and control. Available at: https://www.who.int/cancer/cervical-cancer. Accessed on 11 September 2019.
4. Cay T. Immunhisto-chemical expression of galectin-3 in cancer: a review of the literature. Türk Patoloji Derg. 2012;28(1):1-10.
5. Liu J, Cheng Y, He M, Yao S. Vascular endothelial growth factor C enhances cervical cancer cell invasiveness via upregulation of galectin-3 protein. Gynecol Endocrinol. 2014;30(6):461-5.
6. Jackson P, Blythe D. Immunohistochemical techniques. In: Bancroft JD, Gamble M, editors. Theory and Practice of Histological techniques. 7th ed. New York: Churchill Livingstone. 2012;381-426.
7. Gamble M. The Hematoxylins and Eosin. In: Bancroft JD, Gamble M. Theory and Practice of Histologic techniques. 6th ed. Philadelphia: Churchill Livingstone. 2008:121-34.
8. Bio-Medical Waste Management (Amendment) Rules 2018. New Delhi: Gazette of India, Extraordinary, Part II, Section 3, Subsection (i); 2018. Available at: http://www.egazette.nic.in/WriteReadData/2018/183847.pdf. Accessed on 11 Sep, 2019.
9. Newlaczyl AU, Yu LG. Galectin-3-a jack-of-all-trades in cancer. Cancer Lett. 2011;313(2):123-8.
10. Kim SS, Cho HY, Kang SW, Kim HB, Park SO. Is the expression of p16ink4a and galectin-3correlated with disease progression of cervical neoplasia? Korean J Obstet Gynecol. 2011;54(4):192-8.
11. Punt S, Thijssen VL, Vrolijk J, de Kroon CD, Gorter A, Jordanova ES. Galectin-1, -3 and -9 Expression and Clinical Significance in Squamous Cervical Cancer. PLoS ONE. 2019;10(6): e0129119.
12. Li M, Feng YM, Fang SQ. Overexpression of ezrin and galectin-3 as predictors of poor prognosis of cervical cancer. Braz J Med Biol Res. 2017;50(4):e5356.
13. Ma J, Zhang X, He G, Yang C. The relationship between cervical precancerous lesion galectin-3 and p27 protein expression and clinical prognosis. Oncol Lett. 2018:15:1533-6.
14. Kumar R, Mandal S, Arora P, Mala YM, Khurana N. The expression of p16 and galectin-3 in cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC) uterine cervix. J Obstet Gynaecol. 2021;41(5):785-90.

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