Original Article

Galectin-1 expression in oral squamous cell carcinoma: An immunohistochemical study

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Abstract

Context: Oral squamous cell carcinoma (OSCC) of the head and neck are a heterogeneous group of neoplasms with an increasing rate of mortality and morbidity. OSCCs are characterized by a high degree of local invasiveness and metastasis to cervical lymph nodes but show a lower rate of distant metastasis. Galectin-1 (Gal-1), a β-galactoside-binding lectin, is known to regulate tumor cell growth, angiogenesis, mediate cell-cell or cell-extracellular matrix adhesion and promote cancer cell migration.

Aims: This study aims to evaluate the Gal-1 expression in different clinical stages and histological grades of OSCC.

Settings and Design: Forty histopathologically diagnosed cases of OSCC, including 16 cases of well-differentiated, 18 moderately differentiated and 6 poorly differentiated carcinomas, were included in the study group.

Materials and Methods: The samples were subjected to staining using primary mouse monoclonal antibodies against Gal-1 and visualized using polymer-HRP detection system.

Statistical Analysis: The nonparametric Mann–Whitney U-test and Kruskal–Wallis ANOVA test were used for the statistical analysis.

Results: Gal-1 expression was higher in advanced stages of OSCC, and the results were statistically significant. Immunoexpression of Gal-1 increased with advancing histological grades of OSCC with statistically significant results.

Conclusion: Gal-1 plays an important role in invasion, metastasis and as a prognostic marker.

Keywords: Galectin-1, immunohistochemistry, invasion, metastasis, oral squamous cell carcinoma

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is emerging as a global health problem with 300,000 new cases of oral and oropharyngeal cancers being diagnosed over 5 years.[1] OSCC is the sixth and fifteenth common cancer in men and women, respectively. It involves approximately 94% of all oral malignancies.[2] The highest prevalence and incidence of OSCC is found in the Indian subcontinent.[3] One of the major reasons for the significant mortality related to oral cancer is that 60% of patients present with an advanced

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stage of disease at their initial diagnosis.[4] The etiology of this disease is multifactorial. Considering its clinical view, it is observed in the elderly with low-socioeconomic status. OSCC is able to invade the underlying bone and involve the nerves. Metastatic spread of this disease greatly affects the 5-year survival of patients, and about 50% of patients with head and neck SCC undergo recurrence and metastasis in the first 2 years.[2]

The metastatic spread of tumors continues to be the main barrier to the successful treatment of malignant tumors. About two-thirds of OSCCs are already of substantial size and have clinically detectable metastases to cervical lymph nodes at the time of diagnosis. OSCCs which have metastasized to the regional lymph nodes are more aggressive and have a less favorable prognosis.

Tumor invasion and the process of metastasis is a characteristic of malignant neoplasms. Galectin-1 (Gal-1) is one of the most important lectins participating in malignant tumor development and metastasis.[5] The mechanism by which Gal-1 contributes to cancer progression and metastasis is as follows: it regulates tumor cell growth, triggers the death of infiltrating T cells, suppresses T-cell-derived pro-inflammatory cytokine secretion, mediates cell-cell or cell-extracellular matrix adhesion, is involved in tumor angiogenesis and promotes cancer cell migration.[6] There is evidence that Gal-1 expression increases as cancer cells progress towards a more malignant phenotype and Gal-1 expression levels affect the invasiveness of cancer cells.[6]

The expression of Gal-1 is upregulated in several different human cancers, including breast, gastric, ovary, lung, prostate, colon, nervous system, liver, myeloid tissue and uterine cervix.[5] There is evidence that the mRNA expression levels of Gal-1 are approximately 7-fold higher in OSCC as compared to normal area of the same tissue.[7] Little is known about the prognostic value of Gal-1 in different stages and different histological grades of OSCC. Therefore, the present study aimed to evaluate the Gal-1 expression in different clinical stages and histological grades of OSCC.

MATERIALS AND METHODS

Forty clinically diagnosed and histologically confirmed cases of OSCC were included. The staging of OSCC was done according to the TNM system.[8] An ethical clearance from the institutional ethical committee and informed consent from the patients was obtained for the present study. The cases were graded according to the histological malignancy grading system given by Bryne *et al.* (1989).[9]

Two sections of 4 μm were obtained. One section was placed on egg albumin coated slide for routine hematoxylin and eosin stain. Another section was obtained on aminopropyltriethoxysilane coated slide for immunohistochemistry. These were stained with the Gal-1 antibody at a dilution of 1:100 (Mouse Monoclonal Antibody Galectin-1 (C-8), class-IgG2a, Santa Cruz Biotechnology) using Novolink™ Polymer Detection System. Tonsil tissue was used as a positive control.

Evaluation of immunoexpression of galectin-1

For the quantitative analysis of Gal-1-positive cells, immunostained slides were examined under high power (magnification: ×400) of a light microscope (Olympus CH 20i). A total of 1000 malignant epithelial cells that had invaded the connective tissue were counted in random high power fields. Those which stained positively for Gal-1 were counted among 1000 cells and expressed in terms of positive percentage. We assessed the expression of Gal-1 in the cytoplasm of cells, which had invaded the connective tissue stroma, and the mean was considered as Gal-1 expression for a particular slide. Gal-1 positivity was determined based on the proportion of stained cells and scored from 0 to 4. Score 0 = 0–5% of stained cells, Score 1 = 6%–25% of stained cells, Score 2 = 26%–50% of stained cells, Score 3 = 51%–75% of stained cells and Score 4 ≥ 75% of stained cells.

The nonparametric Mann–Whitney U-test and Kruskal–Wallis ANOVA test was applied for the evaluation of significant differences among the mean values in different groups. Mann–Whitney U test was applied to compare Gal-1 scores with clinicopathological parameters and histological grades. A $P < 0.05$ was considered to indicate statistical significance at 95% of the confidence interval.

RESULTS AND OBSERVATIONS

On comparison of Gal-1 expression with demographic data, we found that Gal-1 expression was higher in males (2.92) than in females (2.75). Mean Gal-1 expression decreased with increasing age groups. The mean expression of Gal-1 was higher in alveolar mucosa (3.25) followed by the tongue (2.93) and then buccal mucosa (2.82) [Table 1]. However, the comparison in the expression of Gal-1 with age, gender and site of OSCC was not found to be statistically significant.

On comparing the expression of Gal-1 score with the nodal status of OSCC, we observed that the mean Gal-1 score increased with regional lymph node involvement

(N0 = 2.63, N = 3.31) and it was found to be highly significant ($P = 0.000$) [Table 1 and Figure 1].

We found 3 cases in Stage I, 21 in Stage II and 16 in Stage III. The levels of Gal-1 protein expression was found to be significantly higher in Stage III (3.31) as compared to Stage I (2.67) and Stage II (2.62) ($P = 0.001$) [Table 1 and Figure 2]. The pairwise comparison showed a statistically significant difference in the Gal-1 expression of Stage II versus III ($P = 0.000$) [Table 2].

Comparison of expression of Gal-1 with histological grades of OSCC showed the highest Gal-1 expression in Grade III (3.83), followed by Grade II (3.00) and Grade I (2.44). This difference in expression was found to be statistically significant ($P = 0.000$) [Table 1 and Figure 3]. The pairwise comparison by the Mann–Whitney U-test showed a significant difference between Grade I versus Grade II ($P = 0.000$), Grade I versus Grade III ($P = 0.000$) and Grade II versus Grade III ($P = 0.000$) [Table 3].

DISCUSSION

Oral cancer is one of the most common cancers in the world. An estimated 378,500 new cases of intraoral cancer are diagnosed annually worldwide. In parts of India, oral cancer represents more than 50% of all cancers and is the most common cancer among males and the third most common cancer among females.[10]

Clinical stage at the time of diagnosis and anatomic region are the most important predictors of survival in HNSCC patients, but the clinical behavior of these tumors varies in different patients and may be attributed to the biological factors concerned in growth and invasion.[11]

Tumor metastasis is a multistep process that includes changes in cell adhesion, increased invasiveness, angiogenesis and evasion of the immune response. Many lectins are involved in metastasis[12] of which Gal-1 has been shown to contribute to all these processes.[13] There is evidence that Gal-1 expression increases as cancer cells progress towards a more malignant phenotype and Gal-1 expression levels affect the invasiveness of cancer cells.[6] A change in the proteolytic degradation of adjacent tissue is required

Table 1: Association of Gal-1 expression and Clinicopathological parameters in oral squamous cell carcinoma

Parameters		Number of patients	Mean Gal-1 score	*P*
Gender	Male	36 (90%)	2.92	0.612
	Female	4 (10%)	2.75	
Age	≤ 40 years	8 (20%)	3	0.552
	40-60 years	30 (75%)	2.90	
	≥ 60 years	2 (5%)	2.50	
Site	Buccal mucosa	22 (55%)	2.82	0.393
	Tongue	14 (35%)	2.93	
	Alveolar Mucosa	4 (10%)	3.25	
Tumor size	T1	3 (7.5%)	2.67	0.484
	T2	37 (92.5%)	2.92	
Lymph node metastasis	N0	24 (60%)	2.63	0.000
	N1	16 (40%)	3.31	
Stages	Stage I	3 (7.5%)	2.67	0.001
	Stage II	21 (52.5%)	2.62	
	Stage III	16 (40%)	3.31	
Grades	Grade I	16 (40%)	2.44	0.000
	Grade II	18 (45%)	3	
	Grade III	6 (15%)	3.83	

Table 2: Pair wise comparison of Gal-1 score among TNM stages by Mann-Whitney U Test

Stage I Vs. II	*p*=0.876
Stage I Vs. III	*p*=0.074
Stage II Vs. III	*p*=0.000 *

Table 3: Pair wise comparison of Gal-1 score among Histopathological grades by Mann-Whitney U Test

Grade I Vs. Grade II	*p*=0.000*
Grade I Vs. Grade III	*p*=0.000*
Grade II Vs. Grade III	*p*=0.000*

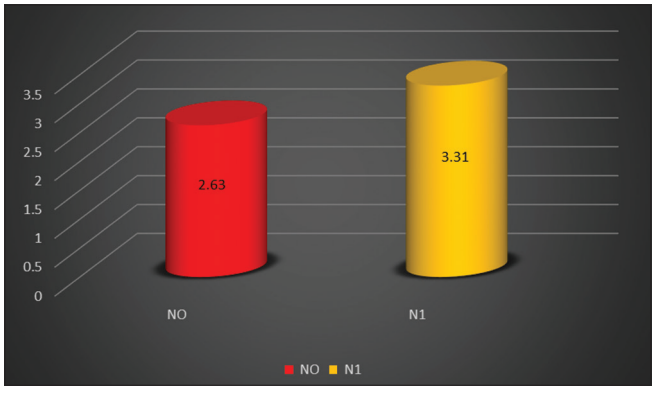

Figure 1: Comparison of galectin-1 score with Nodal status by Mann–Whitney U-test

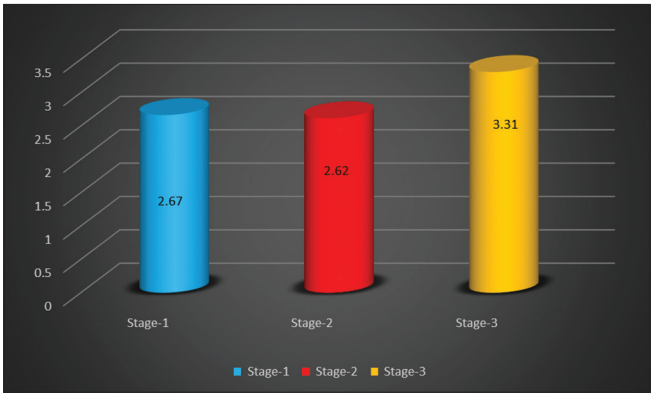

Figure 2: Comparison of galectin-1 score with TNM stages of oral squamous cell carcinoma by Kruskal–Wallis ANOVA

during tumor invasion. Gal-1-increased tumor invasion is related to the reorganization of the actin cytoskeleton and the upregulation of MMP expression.[6] There is a evidence that high Gal-1 expression can be used as a poor prognostic marker for tumors.[14]

In the present study, we assessed the immunoexpression of Gal-1 and correlated it with the clinicopathological parameters of OSCC.

Immunohistochemical analysis of Gal-1 in malignant epithelial tumor cells that had invaded the connective tissue stroma demonstrated an overexpression of this protein in all cases of OSCC studied, suggesting that these molecules play an effective role in tumor invasion and progression.

On comparison of the immunoexpression of Gal-1 and the demographic data, we did not find statistically significant results. These findings were in concordance with those of Zhong *et al*.,[13] Ding *et al.* (2009).[15] These findings could be attributed to the fact that the current study is not an epidemiological type of the study; hence, the limited number of the involved cases preclude definitive clinical findings.

In the present study, we found Gal-1 expression was significantly raised in patients with positive lymph node status ($P = 0.00$). Similar results were reported by Noda *et al*.[16] On the contrary, Zhong *et al*.[13] did not find any correlation between Gal-1 expression and regional lymph node metastasis. Gal-1 contributes to change in cell adhesion and increased invasiveness by upregulation of MMPS; hence, we hypothesized that this may contribute to lymph node metastasis.[6]

On comparison of Gal-1 expression with clinical stages, the Gal-1 expression score was higher in advanced clinical stages, and the difference was found to be statistically significant. This was in contrast to the findings of Noda *et al*.,[16] Zhong *et al*.,[13] Ding *et al.* (2008),[15] who did not find a correlation between Gal-1 expression and clinical stage of OSCC.

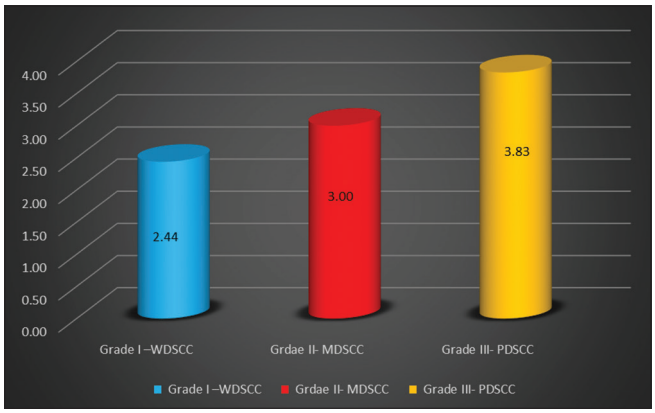

Figure 3: Comparison of galectin-1 scores with Histopathological grades of oral squamous cell carcinoma by Kruskal–Wallis ANOVA

Gal-1 expression was compared with histological grades of OSCC; we observed that the mean Gal-1 expression score gradually increased as the tumor progressed from Grade I to Grade II to Grade III and was also found to be statistically highly significant ($P = 0.000$) [Figures 4-6]. Pairwise intergroup comparison showed expression of Gal-1 was statistically significantly higher in Grade III than in Grade II and Grade I, confirming the hypothesis as previously mentioned, that Gal-1 expression has a significant role in tumor invasion and progression.

Our results confirmed the findings of Noda *et al*.,[16] Zhong *et al*.,[13] Ding *et al.* (2008),[15] who found statistically significant correlation between Gal-1 immunostaining and tumor grade of differentiation and also concluded that higher Gal-1 protein expression indicates a poorer differentiation grade of cancerous tissue and related to poor prognosis.

Greer P *et al.* observed that the Gal-1 is expressed by normal oral keratinocytes (NOK) and OSCC cell lines *in vitro*. Small molecular inhibitor (OTX008) decreases the cell viability of OSCC and NOK cells in a dose-dependent manner; however, this effect is reduced by higher endogenous levels of Gal-1.[17]

In the present study, Gal-1 expression was noted both in the tumor cells and also in the adjacent stromal cells such as fibroblasts and inflammatory cells. The expression of Gal-1 in the nucleus, as well as the membrane of the cells, was observed in some cases. The reason is that Gal-1 is present in cell nuclei and cytosol and also translocates to the intracellular side of cell membranes. Although Gal-1 lacks a secretion signal sequence and does not pass through the endoplasmic reticulum/Golgi pathway, it is secreted and found on the extracellular side of all cell membranes as well as in the extracellular matrices of various normal and neoplastic tissues.[18]

We also observed that the intensity of the staining was strong in Grade III, moderate in Grade II and weak in Grade I. This finding suggests that the Gal-1 protein expression level increases with increasing grades of OSCC, and it may serve as a candidate marker for pathologic differentiation grade of OSCC.

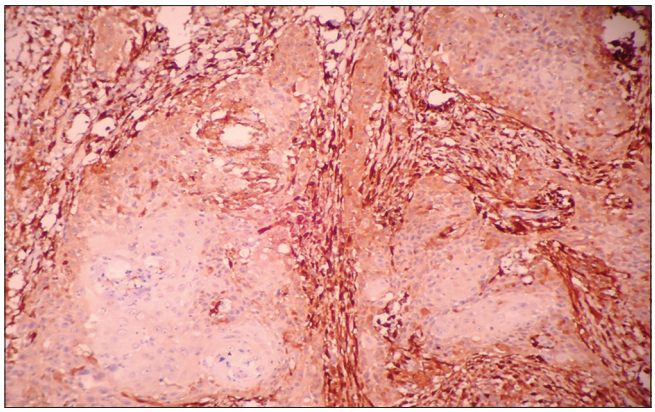

Figure 4: Galectin-1 expression in well-differentiated squamous cell carcinoma (×100)

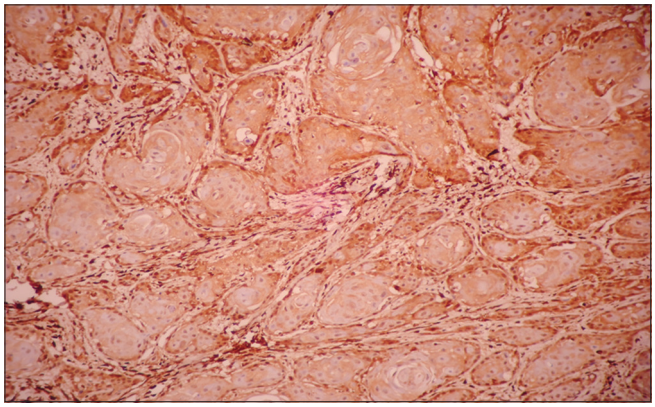

Figure 5: Galectin-1 expression in moderately differentiated squamous cell carcinoma (×100)

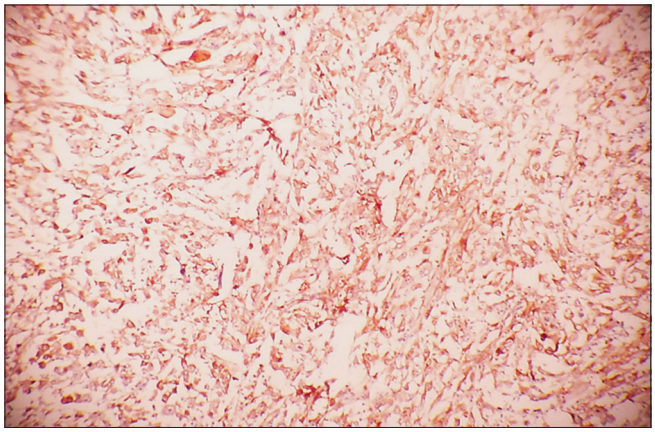

Figure 6: Galectin-1 expression in poorly differentiated squamous cell carcinoma (×100)

The role of Gal-1 in tumor invasion opens a new avenue for developing therapeutic approaches to inhibit tumor metastasis.[6] Gal-1 is involved in the mechanisms of tumor immune escape. Gal-1, a negative regulator of T-cell activation and survival, plays a pivotal role in promoting escape from T cell-dependent immunity, thus conferring the immune privilege of tumors by modulating the survival or polarization of effector T cells.[19] The blockade of immunosuppressive Gal-1 *in vivo* promotes tumor rejection and stimulates the generation of a tumor-specific T cell-mediated response in syngeneic mice. Gal-1 signaling in activated T cells constitutes an important mechanism of tumor-immune escape and that blockade of this inhibitory signal can allow for and potentiate effective immune responses against tumor cells, with profound implications for cancer immunotherapy.[20]

CONCLUSION

In the present study, the upregulation of Gal-1 immunoexpression was seen in OSCC. Gal-1 expression in tumor cells with regional lymph node metastasis was significantly higher than in those without metastasis. The Gal-1 expression also correlated with the clinical stages of OSCC. Thus, Gal-1 can be considered as a strong prognostic factor for the locoregional spread and clinical behavior of OSCC. As Gal-1 expression correlated significantly with histological grades of OSCC, it may serve as a candidate marker for pathologic differentiation grade of OSCC.

Gal-1 is not only a prognostic marker but also an ideal therapeutic target. The role of Gal-1 in tumor invasion opens a new avenue for developing therapeutic approaches to inhibit tumor metastasis.[6] When considering the potential therapeutic use of Gal-1 inhibitors, however, the potential antitumor response of Gal-1 (i.e., role in tumor immune escape) must be taken into account and attempts must also be made to limit inhibitor interference with physiological Gal-1 function.[17] In the future, multicentric studies with large sample sizes are required to positively correlate Gal-1 expression with clinical stages, histological grades and survival rate in OSCC patients.

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Conflicts of interest
There are no conflicts of interest.

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