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IMMUNOHISTOCHEMICAL EXPRESSION OF GALECTIN- 1 IN YOUNG AND OLD PATIENTS OF ORAL SQUAMOUS CELL CARCINOMA

Dental Science

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ABSTRACT

Objectives: The aim of the present paper was to evaluate the expression of Galectin -1 in young and old patients of oral squamous cell carcinoma.
Materials and Methods: Paraffin- embedded blocks of 15 diagnosed cases of OSCC in young and old age groups each were selected for the study. They were stained with Galectin-1 antibody and microscopically examined. Data was analysed by unpaired t test.
Results: In this study, both young and old age groups showed a marked male predilection and the tongue was the commonest site for younger age group while buccal mucosa and alveolar mucosa were mostly involved in the older group. Galectin -1 expression in younger and older age groups of OSCC didn't show any significant difference.
Conclusion: Immunohistochemical study showed that percentage of positive cells and intensity of staining was more in older than in younger patients. However, there was no stastistically significant difference in Galectin-1 expression quantitatively.

KEYWORDS:

galectin – 1,oral squamous cell carcinoma, old patients, young patients.

Introduction :

Oral squamous cell carcinoma (OSCC) is the commonest malignant tumor of the oral cavity, accounting for more than 90 % of all oral malignancies. The incidence of oral cancer parallels the longevity, multiplicity and intensity of carcinogenic exposure. Therefore, the peak incidence tends to arise beyond the 5th decade of life.[1] Conventionally, OSCC is commonly seen in men usually above 40 years of age [2] and only about 0.4 to 3.9 % of the patients affected by OSCC are younger than 40 years.[3] However, epidemiologic analysis has shown disproportionate increase of head and neck squamous cell carcinoma incidence in a younger age group (younger than 45 years old) compared to patients above 45 years .[4] It has been suggested that oral cancer in young patients may be a distinct disease entity on the basis of different biological behaviour and etiological factors. [1]

The general view is that OSCC among young patients is more aggressive and has a worse prognosis.[5] However, in a few studies it has been found that the survival rate of patients below 40 years is better than that of patients above 40 years. [6] The lag time period between exposure of carcinogen initiation and OSCC development in young patients may be short compared to the classical lag time period in older OSCC patients.[4]

Galectin-1 is the first discovered protein in the Galectin family, which is a family of β-galactoside binding lectins with related consensus amino acid sequences and carbohydrate specificity that has been linked to various physiologic and pathologic processes.[7] It contributes to different events associated with inflammation and cancer biology, including tumor transformation, cell cycle regulation, apoptosis, cell adhesion and migration.[8]

The mechanism by which Galectin -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 proinflammatory cytokine secretion, mediates cell-cell or cell extracellular matrix adhesion is involved in tumour angiogenesis, and promotes cancer cell migration.[9] It has been found that Galectin proteins are overexpressed in oral squamous cell carcinoma tumor cells and cancer associated stromal cells. [7] Very few studies have analysed the difference in the immunohistochemical expression of Galectin -1 in young and old patients of OSCC. Studies regarding markers of cell cycle progression, angiogenesis and apoptosis comparing the young and old are sparsely available in the literature. Since Galectin -1 contributes to all these processes , the aim of this study was to assess and compare the immunohistochemical expression of Galectin-1 in young and old patients of oral squamous cell carcinoma.

Materials and Methods :

The study sample comprised two groups of patients from both genders. The patients who were 40 years and below were considered as the "Younger age group" and the patients who were 41 years and above were considered as "Older age group." Paraffin- embedded blocks of 15 diagnosed cases of OSCC in younger and older age groups each were selected for the study and graded according to Bryne's Grading.[10] From the 30 paraffin blocks, two sections of 4 µm thickness were obtained. One section was placed on egg albumin coated slide for routine Hematoxylin and eosin stain. Another section was obtained on amino propyl triethoxy silane (APES) coated slide for immun ohistochemistry. These were stained with Galectin-1 monoclonal antibody using Novolink TM Polymer Detection System (Santa cruz biotechnology).

Firstly, slides were deparaffinised, then were treated with one change each of 100 % ethanol followed by graded ethanol 90 % and 70 % for 5 min each and rinsed with distilled water. Antigen retrieval was done in a pressure cooker and cooling was done for 45 min. Endogenous peroxidase activity was blocked by incubating sections with peroxidase blocking solution for 5 min. Excess was wiped off and sections were washed with phosphate buffer saline (PBS). Then, the sections were blocked with protein block for 5 min. Sections were incubated with primary antibody (Galectin-1 antibody) for 7 h. Incubation was performed in a sealed humidifying chamber at room

temperature to prevent air-drying of the tissue sections for 1 h. Then they were placed in a refrigerator for 6 h at 4°c. After this, the slides were rinsed with PBS for 5 min followed by incubation with Post Primary Block for 30 min and then rinsed with PBS solution twice for 5 min each. Sections were then covered with Novolink Polymer secondary antibody for 30 min at room temperature in the humidifying chamber, rinsed with PBS solution twice for 5 min each. This was followed by incubation with freshly prepared chromogen solution of 3,3 Diaminobenzidine (DAB) for 10 min Sections were rinsed with PBS solution for 5 min and then counterstained with Harri's Hematoxylin for 2 min, dehydrated, cleared and mounted .

Microscopic examination under 400 X magnification of a light microscope (Olympus CH 20i) was done. 1000 cells were counted in random high power fields and positively stained cells were expressed in terms of percentage.

Statistical analysis :

Statistical analysis was performed using unpaired- t test by using software SPSS version 20. When the 'p' value was less than 0.05 the difference was regarded as statistically significant.

Results :

In this study, both younger and older age groups showed a marked male predilection (Table1). The tongue was the commonest site for younger age group while buccal mucosa and alveolar mucosa were mostly involved in the older group (Table 2). In both the groups, most commonly diagnosed cases were moderately differentiated squamous cell carcinoma (Table 3 and Figure 1). The mean value in young patients was 594.20 and 614.07 in older patients with a standard deviation (SD) value of 159.295 and 109.544 respectively. We found that percentage of positive cells and intensity of staining was more in older than in younger patients (Figure 2). Galectin -1 expression in younger and older age groups of OSCC did not show any significant difference statistically (Table 4).

Table 1 : Gender distribution in Young and Old Patients –

Gender	Young < 40yrs	Old > 40yrs
Male	11	12
Female	4	3

Table 2 :Site distribution in Young and Old Patients -

Site	Young < 40yrs	Old >40yrs
Buccal mucosa	5	8
Tongue	8	2
Alveolar Mucosa	2	4
Palate	0	1

Table 3 : Histological Grades Of Oral Squamous Cell Carcinoma -

Grades	Young < 40yrs	Old >40yrs
Well	5	4
Moderate	9	8
Poor	1	3

Figure 1 : Histological Grades of Oral Squamous cell carcinoma-

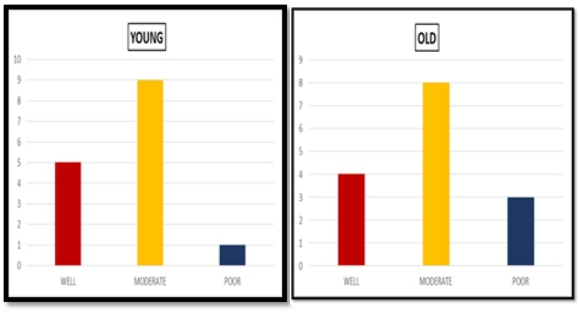
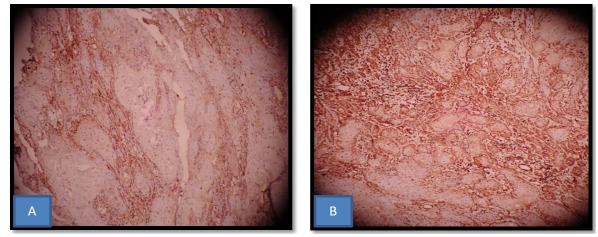


Table 4 : Galectin -1 expression in Young and Old age Group -

	Group	N	Mean	SD	t value	P value
POSITIVE	Young	15	594.20	159.295	0.338	**0.38 (NS)**
	Old	15	614.07	109.544		

SD – Standard deviation ; NS- Non significant ; t – Unpaired t test

Figure 2: Galectin -1 expression in young and old age groups (original magnification 10x) A] Young B] Old.



Discussion :

In India, OSCC represents more than 45 % of all malignancies reported and every year over 1,00,000 new cases of OSCC are registered. Thus, it may contribute to 25 % of all cases of new cancer.[11] It is the sixth most common cancer in the world.[12] OSCC is predominantly seen in middle and old age and is a rarity in those below 45 years.[13] However, alarmingly increasing incidence has been noticed in the younger age group. The majority of oral cancers involve tongue, oropharynx and floor of mouth. A shorter duration of exposure to environmental carcinogens and lack of pre-existing lesions in younger patients suggests the possibility of different molecular mechanisms in the two groups. 4 Siegelmann- Danieli et al. [14] reported that tumors which developed in the absence of tobacco or alcohol occurred more frequently in young patients and that patients at any age who developed disease without these risk factors may have a worse outcome, reinforcing different pathological behaviour.

In this study, patients in both younger and older age groups showed a marked male predilection. The predominance of males in either age group is primarily due to risky oral habits (tobacco, alcohol among males) among Indians. We found that the tongue was the commonest site for younger age group while buccal mucosa and alveolar mucosa were mostly involved in the older group. Most of the patients among young and old age group were diagnosed with Moderately Different iated Squamous Cell Carcinoma (MDSCC) followed by Well Differentiated Squamous Cell Carcinoma (WDSCC) and Poorly Differentiated Squamous Cell Carcinoma (PDSCC).

OSCC is characterized by a high degree of local invasiveness and high rate of metastasis to cervical lymph nodes. We found that the expression of Galectin -1 was noted in tumor cells and adjacent stromal cells like fibroblasts and inflammatory cells. It was observed that the immunohistochemical expression of Galectin- 1 was more intense in poorly differentiated squamous cell carcinoma than the other grades of OSCC.[10] This result is in accordance with studies by Lai-ping Zhong et. al who found that Galectin-1 is negatively correlated with pathologic differentiation grade in oral squamous cell carcinoma.[7]

In the present study, Galectin -1 expression in young and old age groups of OSCC did not show any significant difference (Table 4). Similarly, a study done by Yu-Mei Ding et al 2009[15] also showed that there was no correlation of Galectin -1 expression with demographic data. We observed that the intensity of staining was more in older than in younger patients. However, there was no statistically significant difference in Galectin-1 expression quantitatively. Our findings were in accordance with researchers who studied p53 and Ki-67 expression (which are markers of proliferative activity) and found that there was no statistically significant difference between young and old patients with OSCC. Since there is less effect of extrinsic carcinogenic factors in young patients, the role of intrinsic factors such as genetic and immunological risk-factors should be extensively investigated in such patients.

Conclusion :

Our study revealed that immunohistochemical expression of Galectin -1 in young and old patients of OSCC did not show significant difference. Since, it was a unicentric study, we need to study with multiple markers and a larger sample size included in the study to further confirm the results.

References :

1] Acharya S, Tayaar AS. Analysis of clinical and histopathological profiles of oral squamous cell carcinoma in young indian adults: A retrospective study. J Dent Sci.

2012; 7 (3): 224-230.
2] Sasaki T, Moles DR, Imai Y, Speight PM. Clinico-pathological features of squamous cell carcinoma of the oral cavity in patients <40 years of age. J Oral Pathol Med. 2005; 34(3): 129-33.
3] Syed Mukith R, Ahmed Mujib BR. Histopathological correlation of oral squamous cell carcinoma among younger and older patients. J Oral Maxillofac Pathol. 2014; 18(2):183-188.
4] Monsjou HS ,Wreesmann VB, Brekel MW, Balm AJ. Head and neck squamous cell carcinoma in young patients. Oral Oncol. 2013;49 (12):1097-1102.
5] Kuriakose M, Sankaranarayan M, Nair MK, Cherian T, Sugar C, Scully C, et al. Comparison of oral squamous cell carcinoma in younger and older patients in India. Oral Oncol Eur J Cancer. 1992; 28(2): 113-120.
6] Udeabor SE, Rana M, Wegener G, Gellrich NC, Eckardt AM. Squamous cell carcinoma of the oral cavity and the oropharynx in patients less than 40 years of age: a 20-year analysis. Head Neck Oncol. 2012 ; 4: 28.
7] Lai-Ping Zhong, Kui-jie Wei, Xiao Yang, Hong–ya Pan, Dong-Xia Ye, Li-zhen Wang, Zhi-yuan Zhang. Overexpression of Galectin-1 is negatively correlated with pathologic differentiation grade in oral squamous cell carcinoma. J Cancer Res Clin Oncol. 2010; 136 (10):1527-1535.
8] Rabinovich GA. Galectin –1 as a potential cancer target. Br J Cancer. 2005; 92: 1188-1192.
9] Ming-Heng Wu, Tse-Ming Hong, Hui-Wen Cheng. Szu-Hua Pan,Yu-Ray Liang,Hsiao-chin Hong, Wei-Fan Chiang. Galectin-1 Mediated Tumour Invasion and Metastasis, Up-Regulated Matrix Metalloproteinase Expression, and Reorganized Actin Cytoskeletons. Mol Cancer Res. 2009; 7(3): 311-318.
10] Bryne M, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol.1992;166 (4) : 375-81.
11] Warnakulasuriya S. Global Epidemiology of oral and oropharyngeal Cancer. Oral Oncol. 2009 ;45 (4-5): 309-316.
12] Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer. 1993; 54(4): 594-606.
13] Llewellyn CD, Johnson NW, Warnakulasuriya KA. Risk factors for squamous cell carcinoma of the oral cavity in young people--a comprehensive literature review. Oral Oncol. 2001; 37(5): 401-418.
14] Siegelmann-Danieli N, Hanlon A, Ridge JA, Padmore R, Fein DA, Langer CJ. Oral tongue cancer in patients less than 45 years old: institutional experience and comparison with older patients. J Clin Oncol. 1998;16(2):745-53.
15] Yu-Mei Ding, Ji –Hua Dong, Li –Li Chen,Han-Dong Zhang. Increased expression of Galectin -1 is associated with human oral squamous cell carcinoma development. Oncol Reports. 2009;21: 983-987.