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Study on immunoexpression of beta catenin and Ki 67 in oral squamous cell carcinomas

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***Abstract*---**Cancer is a global health problem. It is the third most common cause of death.1 Among the malignant lesions of the oral cavity squamous cell carcinoma represents the most common cancer. It comprises about 90% of oral cancers.2 To elucidate the expression of immunohistochemical localization of β-catenin, To evaluate proliferative index (ki 67) in oral squamous cell cancers. To stusdy the correlation between β-catenin expression, ki 67 expression and tumor differentiation. Comparative case control study. The study was done in Department of Pathology and E.N.T in a tertiary care hospital. 1 year. patients with signs and symptoms of oral squamous cell carcinoma and investigated in a tertiary care hospital setting. A total of 104 (84 Cases and 20 Controls) were included in the study. Biopsy specimens received to pathology department where gross examination of the specimens was done. Aberrant expression of β-catenin in different histological grades is statistically significant. The p-value is .001361. Aberrant expression of β-catenin increased with increase in histological grade. It can be concluded that β-catenin is a significant factor in predicting the histological grade in patients with SCC. Analysing the expression patterns and locations of β-catenin is useful

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with other previously determined clinicopathological indices in determining prognosis of OSCC.

***Keywords*---**β-catenin, Aberrant expression, oral squamous cell carcinomas.

Introduction

Cancer is a global health problem. It is the third most common cause of death.[1] Among the malignant lesions of the oral cavity squamous cell carcinoma represents the most common cancer. It comprises about 90% of oral cancers.[2] OSCC ranks first in males in Indian subcontinent. Early diagnosis improves patient survival and decrease mortality rates significantly.[3] Risk factors of oral SCC includes tobacco or betel quid chewing, regular drinking of alcoholic beverages, high-risk human papilloma virus genotypes, diet low in fresh fruits and vegetables.[4] Tobacco use has been estimated to be responsible for 90% of oral cancers in India.[5] Most commonly involved anatomic location is tongue.[2] Presence of metastasis to cervical lymph nodes and distant sites is an important prognostic factor in OSCC. Elucidation of various molecular mechanisms involved in the development and maintenance of oral tissues provides insight into the etiology of OSCC. It also helps to identify novel therapeutic targets.[6]

SCC is immunohistochemically labelled by the pan-cytokeratin stain (AE1/AE3) and high molecular weight cytokeratin complex 34βE12, CK5/6 and P63. The Wnt signaling pathway includes genes for various proteins which are involved in functions like growth, proliferation and cellular differentiation.[7] β-catenin is also a part of wnt pathway. It is an intracellular protein that is an integral component of the cadherin-mediated cell-cell adhesion and a downstream transcriptional activator in a Wnt signal transduction pathway.[8,9] Cell proliferation is considered one of the most important mechanisms in oncogenesis.10 In OSCC, the increased Ki-67 index has been correlated with poor survival, high degree of malignancy and histological grading. It is also associated with increased risk of recurrence.[11] The present study is undertaken to study the expression of β-catenin in OSCC in correlation to grades of differentiation and in comparision to ki 67 proliferation index.

Objective

- To elucidate the expression of immunohistochemical localization of β-catenin,
- To evaluate proliferative index (ki 67) in oral squamous cell cancers.
- To stusdy the correlation between β-catenin expression, ki 67 expression and tumor differentiation.

Material and Methods

Study Design

Comparative case control study.

Study area

The study was done in Department of Pathology and E.N.T in a tertiary care hospital.

Study Period

1 year.

Study population

Patients with signs and symptoms of oral squamous cell carcinoma and investigated in a tertiary care hospital setting.

Sample size

A total of 104 (84 Cases and 20 Controls) were included in the study.

Sampling method

Simple Random sampling method.

Inclusion criteria

- Biopsy specimens of histopathologically proven OSCC.
- Specimens from all age groups and both sexes were included.

Exclusion criteria

- Benign lesions of head and neck.
- Biopsies from patients with history of head and neck irradiation.
- Biopsies with extensive areas of necrosis or fibrosis.

Ethical consideration

Institutional Ethical committee permission was taken prior to the commencement of the study.

Study tools and Data collection procedure

Biopsy specimens received to pathology department where gross examination of the specimens was done. Specimens were fixed in 10% neutral buffered formalin and then processed in automated tissue processor and embedded in paraffin wax. 4-5 microns sections were taken and stained with haematoxylin and eosin and were examined microscopically. 84 samples reported as squamous cell carcinoma of the oral cavity were taken for immunohistochemical staining with β-catenin and ki-67. 10 cases of normal squamous epithelium, 10 cases of papilloma were also subjected to immunohistochemistry for β-catenin and ki-67 which acted as controls.

Statistical Analysis

The data was collected, compiled and compared statistically by frequency distribution and percentage proportion. Quantitative data variables were expressed by using Descriptive statistics (Mean ± SD). Qualitative data variables were expressed by using frequency and Percentage (%).

Observations and Results

Table 1
Age distribution in present study

AGE RANGE	NUMBER OF CASES
20 – 30 years	04
31 to 40 years	14
41 to 50 years	17
51 to 60 years	20
61 to 70 years	26
71 to 80 years	03

Table 2
Sex distribution of cases in present study

SEX	NUMBER OF CASES
Male	62
Female	22

Table 3
Distribution of OSCC cases according to the grade of differentiaton

GRADES OF SQUAMOUS CELL CARCINOMA	NUMBER OF CASES
WELL DIFFERENTIATED SCC	51
MODERATELY DIFFERENTIATED SCC	27
POORLY DIFFERENTIATED SCC	06

Table 4
Regional distribution of cases in present study

SITE OF THE LESION	NUMBER OF CASES
Buccal mucosa	17
Hard palate	07
Tongue	50
Lip	2
Floor of mouth	05
Gingiva	04

Table 5
Normal and aberrant expression of beta catenin in present study

GRADE OF DIFFERENTIATION	TOTAL CASES	NORMAL EXPRESSION	ABERRANT EXPRESSION
WDSCC	51	39	12
MDSCC	27	07	20
PDSCC	06	02	04

Aberrant expression of β-catenin in different histological grades is statistically significant. The p-value is .001361. Aberrant expression of β-catenin increased with increase in histological grade. 23.5 % cases of WDSCC, 65 % cases of MDSCC and 83% of PDSCC showed aberrant expression.

Table 6
Beta catenin expression in WDSCC in present study

EXPRESSION		NO OF CASES	PERCENTAGE
NORMAL EXPRESSION	MEMBRANOUS	39	76 %
ABERRANT EXPRESSION	WEAK MEMBRANOUS	05	10 %
	CYTOPLASMIC	07	14 %
	NUCLEAR	00	00 %

Table 7
Beta catenin expression in MDSCC in present study

EXPRESSION		NO OF CASES	PERCENTAGE
NORMAL EXPRESSION	MEMBRANOUS	07	26 %
ABERRANT EXPRESSION	WEAK MEMBRANOUS	04	15 %
	CYTOPLASMIC	16	59 %
	NUCLEAR	00	00 %

Table 8
Beta catenin expression in PDSCC in present study

EXPRESSION		TOTAL NO OF CASES	PERCENTAGE
NORMAL EXPRESSION	MEMBRANOUS	02	33 %
ABERRANT EXPRESSION	WEAK	00	00 %

	MEMBRANOUS		
	CYTOPLASMIC	03	50 %
	NUCLEAR	01	17 %

Table 9
Grade of differentiation and their Ki 67 mean proliferative index

GRADE OF DIFFERENTIATION	< 30 %	30 – 50 %	>50 %	MEAN PROLIFERATIVE INDEX
WDSCC(51)	42	08	1	22.07 %
MDSCC(27)	05	17	05	34.2 %
PDSCC(06)	0	2	4	68.4 %

Discussion

OSCC results from multistep accumulation of heterogenous genetic changes in squamous cells. These changes progressively increase the ability of transformed cells to proliferate and invade. The heterogeneity of these changes explain why tumors of same clinical stage and localization often show significant differences in the clinical outcomes and patient responses. Early-to-moderate-stage OSCC (AJCC stages I-III) is most often treated surgically and sometimes with radiotherapy. Radiotherapy is sometimes ineffective because some cancer cells are not radiosensitive. Chemotherapy in the post-operative period is reserved for patients who have multiple metastatic deposits in lymph nodes and/or those with extracapsular tumor spread. In advanced (stage IV) disease, multidisciplinary non-surgical approaches are being used to improve disease control, prolong survival, and maintain an acceptable quality of life.[5]

OSCC remains one of the most difficult malignancies to control because of its high propensity for local invasion and cervical lymph node dissemination, which is responsible for the poor clinical prognosis, with a 5- year survival rate of only about 50 percent, even with the latest advances in the treatment. This is due to patients dying from metastatic disease despite being diagnosed at an early stage. Detection of occult metastases is difficult, therefore application of prognostic markers in primary diagnostic tumour specimens are highly desirable.
Cai zhi et al[12] , Fernanada ferreira et al[13] found that most number of cases of OSCC were seen in 6th decade. Xia yun et al[14] found most number of cases in 6th decade and Partheeban et al[15] in 4th decade. In the present study most of the cases in OSCC were found in 7th decade of life. The finding of present study were similar to Cai Zhi et al and Fernanda Ferreira et al. In present study age of the patients ranged from 25 years to 80 years with maximum number of cases in 7th decade. Risk of OSCC increases with increase in age and most commonly seen in 6th and 7th decades of life.

The present study holds forth a male to female sex ratio of 3.3:1, which correlates with Partheeban et al[15]. Increased incidence in males is attributable to behavioural and life style factors like tobacco chewing and alcoholism. Increase in consumption of alcohol and tobacco is also reason for early development of OSCC in younger ages. Histological grading of present study comprises of 60.7% of

WDSCC, 32.1 % of MDSCC and 7.1% of PDSCC. These findings correlated best with the findings of the studies done by Partheeban et al.[15] with respect to the distribution of samples according to the degree of differentiation of OSCC.

In present study number of WDSCC cases are 51 (n =51). 76.5% (n=39) showed normal expression and 23.5% (n=12) showed aberrant expression. The findings of the present study correlate best with the findings of the studies done by Partheeban et al[15] with respect to β-catenin expression in WDSCC. Most of the WDSCC appear histologically very similar to normal squamous epithelium with individual cell keratinization and well-formed intercellular bridges. Majority of WDSCC in present study shows membranous expression of β-catenin similar to normal squamous epithelium. In present study number of MDSCC cases are 27 (n =27) . 35 % (n = 7) showed normal expression and 65 % (n = 11) showed aberrant expression. The findings of the present study correlate best with the findings of the studies done by Lankesh B Lakshmidevi et al[16] with respect to β-catenin expression in MDSCC. Percentage of cases showing abnormal expression of β-catenin increased in MDSCC compared to WDSCC.

In present study (2017) number of PDSCC cases are 6 (n =06). 17 % (n = 1) showed normal expression and 83 % (n = 05) showed aberrant expression. The findings of the present study correlate best with the findings of the studies done Xia-Yun et al[14] with respect to β-catenin expression in PDSCC. Majority of PDSCC showed aberrant expression of β-catenin in the form of weak membranous expression, increased cytoplasmic and also nuclear expression which is seen in one case of PDSCC in present study. Increased cytoplasmic protein facilitates entry of β-catenin into nucleus thereby increasing cell proliferation. Increased invasiveness and proliferation of cells in PDSCC results in aggressive course and poor prognosis of the tumor. Aberrant expression of β-catenin in different histological grades is statistically significant. The p-value is .001361. Aberrant expression of β-catenin increased with increase in histological grade. 23.5 % cases of WDSCC, 65 % cases of MDSCC and 83% of PDSCC showed aberrant expression. Pattern of aberrant expression in present study (2017) correlated well with Xia- Yun et al[14]

This clearly explains that β-catenin has a role in progression of OSCC from low grade to high grade which is responsible for the aggressive behavior of the tumor. Decrease membranous degradation results in loss of adhesive functions in more aggressive neoplasms. Reduced membranous expression and predominant cytoplasmic localization are prominent among high grade tumors, suggesting stabilization of β-catenin and its role as a signaling molecule. Positive Ki-67 expression in the nuclei of proliferating tumor epithelial cells was found in 100% of OSCC samples (n=84) included in the study. These results are similar to the observations of the study done by Monica et al.[17] The study conducted by Monica et al.[17] over samples from 105 patients with OSCC (n=105) reported a mean proliferative index of 36.65%. In the study conducted by Birajdar et al.[18], samples from 20 patients of OSCC (n=20) were included in the study, and the mean proliferative index was 71.09%. The present study (2017) was conducted on samples from 80 patients of OSCC (n=80), and the reported mean proliferative index is 41.03%.

In the present study, the values for the mean Ki-67 proliferative indices in WDSCC, MDSCC and PDSCC are 22.07%, 34.2% and 68.4% respectively. In the present study(2017), the total number of samples of OSCC studied were 84(n=84). Among the 51 samples of WDSCC(n=51/84) ,82% of the samples(n=42/51) had a low index of proliferation,16% of the samples(n=08/51) had a moderate index of proliferation and 2% of the samples(n=1/51) had a high index of proliferation. Among the 27 cases of MDSCC (n=27/84), 19% of the samples (n=5/27) had a low index of proliferation, 62% of the samples(n=17/27) had a moderate index of proliferation and19% of the samples(n=5/27) had a high index of proliferation. Among the 06 samples of PDSCC(n=06/84),none of the samples(n=0/06) had a low index of proliferation,34% of the samples(n=2/06) had a moderate index of proliferation and 66% of the samples(n=4/06) had a high index of proliferation.

Conclusion

It can be concluded that β-catenin is a significant factor in predicting the histological grade in patients with SCC .analyzing the expression patterns and locations of β-catenin is useful with other previously determined clinicopathological indices in determining prognosis of OSCC.

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