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ORIGINAL RESEARCH

Immunoexpression of WT1 and Ki-67 Gene in Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral cancers contribute to substantial morbidity and mortality, by being the sixth most common cancer worldwide. The most common malignant epithelial neoplasm in the oral cavity is oral squamous cell carcinoma (OSCC), representing over 90% of malignancies of the oral cavity. The remaining include malignant tumors of salivary gland, lymphoreticular disorders, bone tumors, malignant melanomas, sarcomas, malignant odontogenic tumors, and metastatic deposits.

Material and Methods: This is a prospective study conducted at Department of Pathology, Ayaan Insitute of Medical Sciences, Kankamidi from 2019 to 2020. The present study was WT1 and Ki-67 immunoexpression in oral squamous cell carcinoma was conducted on 80 cases of oral squamous cell carcinoma. 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. 80 cases reported as squamous cell carcinoma of the oral cavity were taken for immunohistochemical staining with WT1 and Ki-67.

Results: A total number of 100 cases were studied, the test group included a total of 80 cases of OSCC. 50 cases (n=50/80) were well differentiated squamous cell carcinomas (WDSCC), 20 cases (n=20/80) were moderately differentiated squamous cell carcinomas (MDSCC) and 10 cases (n=10/80) were poorly differentiated squamous cell carcinoma (PDSCC). The Ki-67 LI was calculated for all the cases of OSCC obtained from various intraoral sites and the mode of the extent of proliferation was tabulated. Among the cases showing well differentiation, 57.14% of the cases (n=4/7) had a low extent of proliferation,28.5% of the cases (n=2/7) had a moderate extent of proliferation and 14.28% of the cases (n=1/7) had a high extent of proliferation.

Conclusion: OSCC is one of the most common cancers in India, contributing to substantial morbidity and mortality. The right lateral border of the tongue is the commonest site of OSCC in the present study. The prognosis of patients with Stage 1 of oral tongue SCC, depends on the Ki-67 LI, with a good prognosis being associated with a Ki-67 LI less than 33%. WT1 immunotherapy can be advocated to improve the prognosis of patients with histopathologically proven WDSCC and a Ki-67 LI more than 33%.

Keywords: Immunoexpression, WT1, Ki-67, Oral Squamous Cell Carcinoma.

INTRODUCTION

Oral cancers contribute to substantial morbidity and mortality, by being the sixth most common cancer worldwide. [1] Epidemiological studies of oral cancer showed that Southern Asia has the highest incidence of oral cancer, accounting for 18% of all cancers, followed in close succession by South-east Asia, Western and Central Europe, and South America. [2]

The most common malignant epithelial neoplasm in the oral cavity is oral squamous cell carcinoma (OSCC), representing over 90% of malignancies of the oral cavity. [3] The remaining include malignant tumors of salivary gland, lymphoreticular disorders, bone tumors, malignant melanomas, sarcomas, malignant odontogenic tumors, and metastatic deposits. [4]

Cancers of oral cavity include tumors arising from the mucosal surfaces of the mouth, beginning from the vermillion border of the upper and lower lips and extending upto the palatoglossal folds and the tumors of salivary glands. [5]

OSCC frequently co-exists with epithelial dysplasia in the oral cavity. Oral submucous fibrosis and oral lichen planus are precancerous conditions in which immuno- inflammatory processes are implicated in their cancerous transformation. [6] Malignant transformation of oral precancerous lesions is observed at a frequency of 17.5%, and the risk is seen to increase with increasing degree of dysplasia. [7]

The traditional risk factors for OSCC include tobacco smoking and chewing (90%), betel-quid chewing, diet low in fruit and vegetable and chronic candidiasis and HPV infection. [8] High exposure to ultraviolet light and immunosuppression increases the chance of developing cancer of the lower lip. [9]

A number of rare conditions predispose to the development of oral cancer, such as xeroderma pigmentosum, Fanconi's anaemia, and Bloom's syndrome.[10] It is now established that up to 10% of all cancers have a strong hereditary component. [11]

Squamous cell carcinoma (SCC) is immunohistochemically labelled by the pan- cytokeratin stain (AE1/AE3), High molecular weight cytokeratin complex 34 βE12, CK5/6 and P63. [12]

There are very few studies elucidating the role of WT1 in the pathogenesis of OSCC. The purpose of this study is to document the immunoexpression of WT1 and Ki-67 in 80 cases of OSCC, and to explore the possibility of implementation of WT1 targeted therapy, in the cases with WT1 immunoexpression. [13]

AIMS AND OBJECTIVES

To evaluate WT1 and Ki-67 immunoexpression in histopathologically proven cases of oral squamous cell carcinoma (OSCC).

MATERIAL AND METHODS

This is a prospective study conducted at Department of Pathology, Ayaan Insitute of Medical Sciences, Kankamidi from 2019 to 2020. The present study was WT1 and Ki-67 immunoexpression in oral squamous cell carcinoma, a prospective study was conducted on 80 cases of oral squamous cell carcinoma

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. Cystic lesion of oral cavity. Biopsies with extensive areas of necrosis or fibrosis. Biopsies from patients with history of head and neck irradiation

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. 80 cases reported as squamous cell carcinoma of the oral cavity were taken for immunohistochemical staining with WT1 and Ki-67.

4 cases of hemangioma, 10 cases of papilloma and 5 cases of hamartoma were also subjected to immunohistochemistry for WT1 and Ki-67 which acted as controls.

10 cases of squamous papillomas were consistently negative with WT-1 immunostaining and acted as negative control. 5 cases of hemangioma acted as positive controls. Basal cell layer of epithelium, vascular endothelium and peripheral nerve fibers in the sections also showed cytoplasmic staining; therefore, the positive staining of these cells acted as an internal control.

Immunohistochemical staining of WT1 (6F-H2 mouse monoclonal anti human antibody-Dako) and Ki-67(Mib-1 mouse monoclonal antibody-Biogenex) was done using peroxidase–antiperoxidase method.

EVALUATION OF STAINING

For evaluation of WT1 expression, staining intensity was scored as 0 (negative), 1 (weak), 2 (medium) and 3 (strong). The extent of staining was scored as 0 (0%), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%) and 4+ (76-100%) according to the percentage of the positive staining area in relation to the whole carcinoma area.

Positive- Brown stain in the nucleoli and nucleoplasm of tumor cells considered positive Section of lymph node with reactive hyperplasia used as control.

RESULTS

A total number of 100 cases were studied, the test group included a total of 80 cases of OSCC. 50 cases (n=50/80) were well differentiated squamous cell carcinomas (WDSCC), 20 cases (n=20/80) were moderately differentiated squamous cell carcinomas (MDSCC) and 10 cases (n=10/80) were poorly differentiated squamous cell carcinoma (PDSCC). (Table 1)

The control group included 20 cases, which were benign lesions of oral cavity (5 cases of hemangioma, 5 cases of hamartoma and 10 cases of papilloma).

Table 1: Distribution of OSCC cases included in study according to the grade of differentiation

GRADE OF OSCC	NUMBER OF CASES
WDSCC	62.5% (n=50/80)
MDSCC	25% (n=20/80)
PDSCC	12.5% (n=10/80)

The epithelial nature of poorly differentiated malignancies in the study was proven by positive nuclear immunostaining for P63 protein.

Table 2: Regional distribution of cases in the present study

SITE OF THE LESION	NUMBER OF CASES
Tongue	48
Palate	12
Buccal mucosa	08

Floor of mouth	07
Gingiva	03
Lip	02

Out of the 80 cases included in the study, 48 cases (n=48/80) were obtained from the tongue (right lateral border), 12 cases (n=12/80) from the palate, 8 cases (n=8/80) from the buccal mucosa, 7 cases (n=7/80) from the floor of mouth, 3 cases (n=3/80) from the gingiva and 2 cases (n=2/80) from the lip (Table 2).

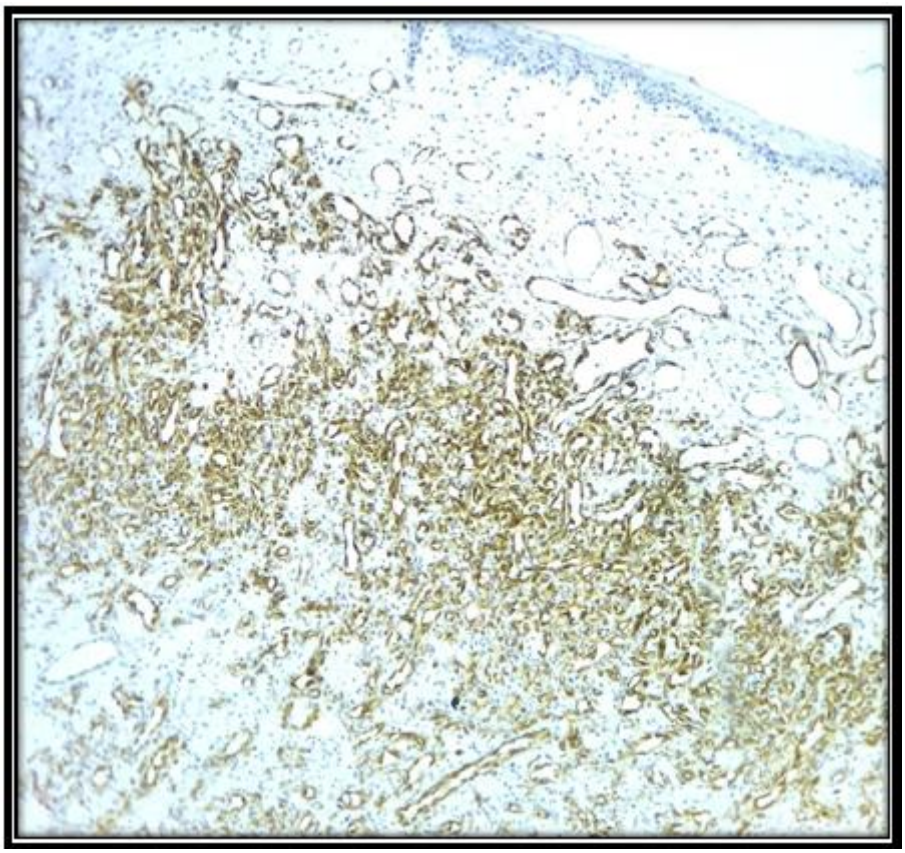

Figure 1: Detection of WT1 protein by immunohistochemistry - WT1 seen decorating the endothelial cells of a hemangioma (control).

Table 3: Grade of differentiation of OSCC and MPI

GRADE OF DIFFERENTIATION	MEAN PROLIFERATIVE INDEX
WDSCC	22.05%
MDSCC	25%
PDSCC	64%

Table 4: Distribution of cases based on grade of differentiation and extent of proliferation

GRADE OF DIFFERENTIATION	EXTENT OF PROLIFERATION (BASED ON KI-67 LI)		
	LOW(<30%)	MODERATE (30-50%)	HIGH(>50%)
WDSCC	78% (n=39/50)	20% (n=8/20)	2% (n=1/50)
MDSCC	45% (n=9/20)	40% (n=8/20)	15% (3/20)
PDSCC	-	30% (n=3/10)	70% (n=7/10)

Table 5: Mode of Ki-67 LI at various intraoral sites of OSCC

SITE	GRADE OF DIFFERENTIATION			MODE
	WDSCC	MDSCC	PDSCC	
TONGUE	33	11	4	<30%
BUCCAL MUCOSA	5	2	1	<30%
PALATE	8	2	2	<30%
FLOOR OF MOUTH	3	4	0	<30%
GINGIVA	-	1	2	>50%
LIP	1		1	<30%

The Ki-67 LI was calculated for all the cases of OSCC obtained from various intraoral sites and the mode of the extent of proliferation was tabulated (Table 5).

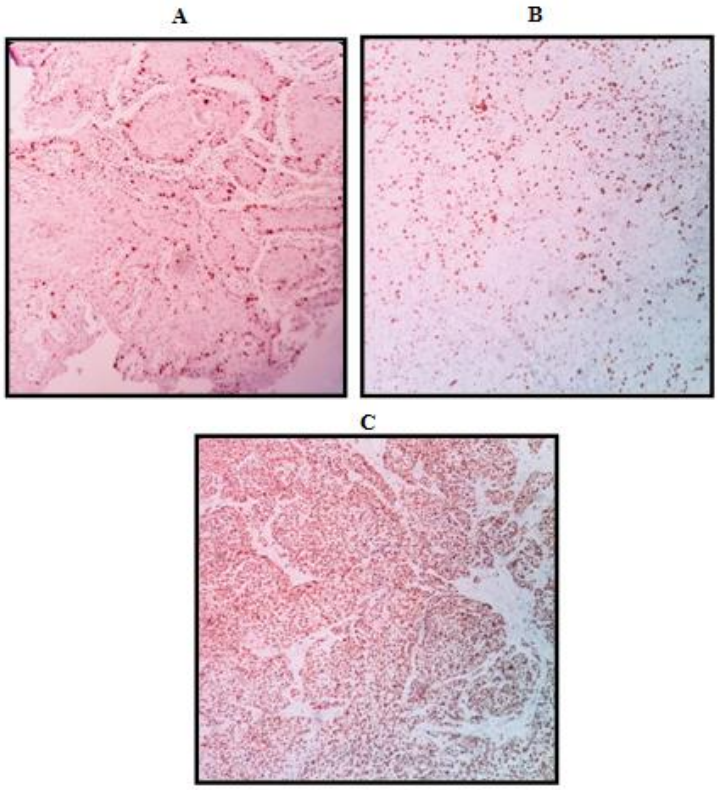

Figure 2: A) Ki-67 immunoexpression (10x) in WDSCC demonstrating a low extent of proliferation, mostly localised to the periphery of tumor islands. B) Ki-67 immunoexpression (10x) in MDSCC demonstrating a low extent of proliferation. C) Ki-67 immunoexpression (10x) in PDSCC demonstrating a high extent of proliferation

Table 6: WT1 expression and Extent of proliferation (based on Ki-67 LI)

EXTENT OF PROLIFERATION	WT1 POSITIVE CASES		
	WDSCC(n=7/10)	MDSCC(n=3/10)	PDSCC
LOW INDEX	57.14% (n=4/7)	33.3% (n=1/3)	0
MODERATE INDEX	28.5% (n=2/7)	33.3% (n=1/3)	0
HIGH INDEX	14.28% (n=1/7)	33.3% (n=1/3)	0

Among the cases showing well differentiation, 57.14% of the cases (n=4/7) had a low extent of proliferation, 28.5% of the cases (n=2/7) had a moderate extent of proliferation and

14.28% of the cases (n=1/7) had a high extent of proliferation (Table 6).

Table 7: Summary of clinical features and Ki-67 LI of patients with WDSCC and positivity of WT1 on immunohistochemistry

AGE and SEX	LOCATION	WT1 STAINING PATTERN	KI-67 LI
30/M	TONGUE	STRONG, 2+	30%
31/M	TONGUE	STRONG, 4+	30%
46/F	TONGUE	STRONG, 4+	5%
32/M	BUCCAL MUCOSA	WEAK, 1+	52%
30/M	BUCCAL MUCOSA	STRONG, 3+	45%
25/F	BUCCAL MUCOSA	STRONG, 3+	45%
50/M	FLOOR OF MOUTH	STRONG, 2+	40%

Table 8: Summary of clinical features and Ki-67 proliferation index of patients with MDSCC and positivity of WT1 on immunohistochemistry

AGE AND SEX	LOCATION	WT1 STAINING PATTERN	KI-67 LI
70/M	TONGUE	MODERATE, 2+	40%
60/M	TONGUE	MODERATE, 2+	55%
80/F	FLOOR OF MOUTH	MODERATE, 1+	25%

Table 7 summarises the clinical features and Ki-67 LI of cases with WDSCC and WT1 positivity on immunohistochemistry. **T**able 8 summarises the clinical features and Ki-67 LI of cases with MDSCC and WT1 positivity on immunohistochemistry.

DISCUSSION

SCC is the primary tumor type in the head and neck region. Oral cancer patients have a high chance of recurrence and/or metastasis, 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. [14] 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 tumor specimens are highly desirable. [15]

It has been shown that WT1 is overexpressed in a number of cancer cells, and the knockdown of WT1 by antisense oligomers could induce mitochondrial damage and then inhibit malignant cell growth. [16] WT1 is a promising target for immunotherapy based on preliminary results from vaccine trials which reveal WT1's untapped potential to induce cancer immunity with minimal side effects. [17]

The present study entails the immunoexpression of WT1 and Ki-67 in oral squamous cell carcinoma. Demographic profile including age, sex, site and incidence was analysed and compared with literature.

Histopathological grading of the cases included in all the following studies is according to the grading system given by Broder, (based on the percentage of immature cells) into WDSCC, MDSCC and PDSCC. In the study by Yusuki Oji et al. (2003), [18] the total number of cases studied were 38 (n=38), out of which 44.7% (n=17/38) cases were WDSCC, 47.3% (n=18/38) were MDSCC and 7.8% (n=3/38) cases were PDSCC. Maximum number of cases in this study were MDSCC. In the study done by Mikami et al. (2013), [19] the total number of cases included in the study was 29 (n=29), out of which 86.2% (n=25/29) were WDSCC, 10.3% (n=3/29) were MDSCC and 3.4 % (n=1/29) were PDSCC. Maximum number of cases in this study.

Yusuki Oji et al. (2003) [18] reported 3 cases (n=3/38) with cytoplasmic positivity for WT1 protein, out of a total number of 38 cases of OSCC included in the study. The percentage positivity of WT1 immunoexpression is 7.8% (n=3/38). Mikami et al. (2013) [19] reported

cytoplasmic expression of WT1 protein in 2 cases (n=2/29), out of a total number of 29 cases of OSCC included in the study. The percentage positivity of WT1 immunoexpression is 6.8% (n=2/29).

Fattahi et al. (2016) [20] reported cytoplasmic immunoexpression of WT1 protein in 3 cases (n=3/45), out of a total number of 45 cases of OSCC included in the study. The percentage positivity of WT1 immunoexpression is 6.6% (n=3/45). In the present study (2017), 10 cases (n=10/80) of OSCC were reported to be positive for the cytoplasmic immunoexpression of WT1 protein, out of a total number of 80 cases. The percentage positivity of WT1 immunoexpression is 12.5% (n=10/80). The findings of the present study correlate best with the findings of the study by Yusuki Oji et al. (2003) [18] with respect to the percentage positivity of WT1 imunoexpression.

Positive Ki-67 expression in the nuclei of proliferating tumor epithelial cells was found in 100% of OSCC cases (n=80) included in the study. This is in close correspondence with the observations of the study done by Mahima et al.(2015). [21]

The study conducted by Dwivedi et al. (2013) includes cases from 15 patients (n=15) of OSCC, and they reported a mean proliferative index of 39.45%. [22] In the study conducted by Birajdar et al. (2014), cases from 20 patients of OSCC (n=20) were included in the study, and the mean proliferative index was 71.09%. [23] The study conducted by Mahima et al. (2015) over cases from 105 pateints with OSCC (n=105) reported a mean proliferative index of 36.65%. [21]

The present study (2017) was conducted on cases from 80 patients of OSCC (n=80), and the reported mean proliferative index is 37.01%, and is similar to the values observed by Mahima et al. (2015). [21] In the study by Birajdar et al. (2014), the mean labeling index among the WDSCC (n=7/20), MDSCC (n=7/20) and PDSCC (n=6/20) was 74.08%, 51.60% and 87.60% respectively. [23] In the study by Mahima et al. (2015), the mean labeling index among the WDSCC (n=35/105), MDSCC (n=35/105) and PDSCC (n=35/105) was 29.84%, 48.10% and 32.01% respectively. [21]

In the present study (2017), the mean labeling index among the WDSCC (n=50/80), MDSCC (n=20/80) and PDSCC (n=10/80) was 22.05%, 25% and 64% respectively. In the present study (2017), the values for the mean Ki-67 proliferative indices in WDSCC, MDSCC and PDSCC are 22.05%, 25% and 64% respectively. The values obtained for cases of WDSCC and MDSCC correlate with the values of mean Ki-67 proliferative indices obtained in the study by Mahima et al. (2015), with them being 29.84% and 48% respectively. [21] The mean proliferative Ki-67 index of cases of PDSCC correlates with the value obtained by Birajdar et al. (2014), with the value being 87.60%. [23]

In the study done by Mahima et al. (2015), an analysis of the Ki-67 proliferation and the intra-oral site of OSCC was done. [21] Ki-67 proliferation of nine different sites of the oral cavity was assessed and its mode was calculated. The tongue (dorsal/ventral aspect), vestibule and floor of the mouth and buccal mucosa with alveolus show low extent of Ki- 67 proliferation (<30%), whereas angle of mandible, alveolar region and buccal mucosa show moderate extent of Ki-67 proliferation (30-50%) and tongue (lateral border), alveolar region and lips show high extent of Ki- 67 proliferation (>50%) and least degree of tumoral differentiation.

In the present study (2017), an analysis of Ki-67 proliferation and intra oral site of OSCC yielded that the tongue (lateral border), buccal mucosa, hard palate and floor of the mouth showed low Ki-67 proliferation (<30%), whereas gingival carcinomas had high Ki-67 proliferation (>50%) and the least degree of tumoral differentiation.

CONCLUSION

OSCC is one of the most common cancers in India, contributing to substantial morbidity and mortality. The right lateral border of the tongue is the commonest site of OSCC in the present

study. The prognosis of patients with Stage 1 of oral tongue SCC, depends on the Ki-67 LI, with a good prognosis being associated with a Ki-67 LI less than 33%. WT1 immunotherapy can be advocated to improve the prognosis of patients with histopathologically proven WDSCC and a Ki-67 LI more than 33%.

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