ORIGINAL ARTICLE

INTENSITY OF WILM'S TUMOR (WT1) IMMUNOMARKER IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

In oral squamous cell carcinoma (OSCC), the nests of tumor cells infilterate the underlying connective tissue of the oral squamous cell epithelium with increasing nuclear mitotic activity. In this study we are determining the role of tumor cells proliferation by evaluating the intensity of WT1 marker in OSCC. Nuclear intensity of Wilm's tumor 1 (WT1) immunomarker was analyzed on sixty eight cases of well differentiated oral squamous cell carcinoma (WD-OSCC), twenty one cases of moderately differentiated oral squamous cell carcinoma (MD-OSCC) and eleven cases of poorly differentiated oral squamous cell carcinoma (PD-OSCC). Thirty seven percent (37 cases) of the cells showed 0 reactivity, thirty seven percent (37 cases) showed 1+ reactivity, twenty two percent (22 cases) showed 2+ reactivity, four percent (4 cases) showed 3+ reactivity. Seventy nine percent (54 cases) of positivity in WD-OSCC (68 cases), nineteen percent (4 cases) positivity in MD-OSCC (21 cases) and forty five percent (5 cases) positivity in PD-OSCC (11 cases). P value 0.024 significantly correlated with the histological grades of OSCC and tumor cell proliferation. The nuclear staining suggested the role of WT1 in cancer nests proliferation suggesting its oncogenic role in oral squamous cell carcinoma.

Key Words: *Oral Squamous Cell Carcinoma, Well Differentiated, Moderately Differentiated, Poorly Differentiated).*

INTRODUCTION

The oral mucosa is the mucous membrane lining the interior of the mouth. It consists of stratified squamous epithelium with an underlying connective tissue known as lamina propria. Any changes indicative of disease are seen as alterations in the oral mucosa, which can reveal systemic conditions such as diabetes, vitamin deficiency, local effects of chronic tobacco or alcohol use.[1,2] Squamous cell carcinoma (SCC) is a malignant neoplasm of the squamous cells that can cause locally destructive growth and distant metastasis. WHO defines SCC as "an invasive epithelial neoplasm with varying degrees of squamous differentiation and a propensity to early and extensive lymph node metastasis, occurring predominantly in alcohol and tobacco using adults in the 5th and 6th decades of life".[3,4] Histopathological grading is an important factor in determining the prognosis of OSCC. The most important and acceptable parameter is 'invasive front grading' in predicting nodal metastasis, local recurrence and survival. Broder's classification of Oral Squamous Cell Carcinoma 1)Well differentiated (Grade-I)-<25% undifferentiated cells, 2)Moderately differentiated (Grade-II)-<50% undifferentiated cells, 3)Poorly differentiated (Grade-III)-<75% undifferentiated cells, 4)Anaplastic/pleomorphic(Grade-IV)->75% undifferentiated cells.[5]

The WT1 was originally isolated as a tumor-suppressor gene that is inactivated in a subset of Wilms'tumor and mutated in the germline of children with genetic predisposition to this kidney neoplasm of childhood. The WT1 gene encodes a zinc finger transcription factor that regulates transcription of growth factor such as PDGF-A and growth factor receptor (IGF-IR) genes and other genes (RAR-·, c-myc and bcl-2).[6,7] WT1 promotes cell growth, migration, inhibition and invasion, inhibits apoptosis and induces tumorigenecity in leukemia.[8] The pathogenic role of WT1 in OSCC has not been studied extensively The aim of the study is to investigate the nuclear intensity by WT1 immunomarker in histological grades of OSCC and its association in tumor cell proliferation.

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METHODOLOGY

The Descriptive study was carried out in Department of Histopathology, Armed Forces Institute of Pathology (AFIP, Rawalpindi) and Ethical approval was taken from the respective institute. The sample size was 100 cases and was calculated using WHO calculator[9] (n= Z2 PQ/ d2, where n = desired sample size, Z = standard normal deviate, corresponding to 95% confidence level, P = proportion in the target population estimated to have a particular characteristic, Q =1-P = proportion in the target population not having the particular characteristics) and d= degree of accuracy required, Z2 =3.8416, P=7% (0.07)[9], Q=1-P, d2=0.0025). The sampling technique was Non Probability convenience sampling.

Hundred (100) paraffin embedded blocks of OSCC, previously and freshly diagnosed at AFIP, Rawalpindi were collected along with the relevant demographic and clinical/radiographical information. Freshly diagnosed cases or archival tissue blocks of OSCC of all the grades were obtained from different hospitals. All age groups, both genders involving the oral cavity diagnosed at AFIP Rawalpindi were included. Poorly fixed specimen, necroed or autolyzed tissue samples and tumors with scanty tissue were excluded and previously treated cases of OSCC were also excluded.

Tissues were prepared for microscopic examination. The tissue processing was start with the fixation of the surgical specimen or biopsy using buffered formalin (10%). All the representative section from the surgical specimen were taken and processed in labeled plastic cassettes with perforated walls. These were then placed in automatic tissue processor Tissue Tek VIP-5 processor. The processing involved dehydration using increasing strengths of alcohol which was replaced by xylene at 38^0C, followed by impregnation of the tissue with molten paraffin wax at temperature of 52-55^0C. Casting or blocking was then carried out using Tissue Tek Embedding Console system, filling metal moulds with fresh molten wax and was allowed to cool. Finally the cooled blocks were trimmed and very thin sections (3-5 microns) were cut using Accu Cut Rotary Microtome SRM 200-1. The sections were picked up on clean and labeled frosted glass slides and were stained in Varistain Multiproy slide stainer. The immunohistochemistry was then applied using the indirect method, It is a two-step method with an unlabeled primary antibody (first layer) reacting with tissue antigen followed by application of secondary labeled antibody (second layer) reacting with primary antibody. This method has adequate sensitivity and second layer antibody can be labelled with a fluorescent dye or enzyme. After high temperature unmasking sections were washed in tris buffered saline (TBS) pH 7.6 for 5 minutes. These were placed in diluted normal serum or normal horse serum for 10 minutes and then incubated with primary antibody. After the application these were washed in TBS buffer for 5 minutes and then incubated in an appropriate biotinylated secondary antibody. Sections were washed in TBS again buffer for 5 minutes. Slides were then incubated in ABC (avidin biotin) reagent and washed in TBS buffer again for 2-5 minutes. Slides were incubated in Diamino benzidine (DAB) or other suitable peroxidase substrate. Finally washed in running tap water and were counterstained with haematoxylin, dehydrated and mounted and observed under microscope.

Immunoreactivity of WT1 was evaluated according to the staining intensity and localization of the tumor cell nuclei and graded 0, 1+, 2+, and 3+, corresponding to absent, focal/weak, moderate, and intense expression. Similar grading scales have previously been used in the immunohistochemical expression studies of WT1.[10,11] The expression pattern was organized into two groups; high expression (2+ to 3+) and low expression (0 to 1+) and their relationship was correlated with histological grade of differentiation.[12]

The information collected in the form of variables on specially designed data collection proforma was analyzed using SPSS version 17.0. Descriptive statistics were used to describe the data. Mean and standard deviation was used to describe numeric variables like age and gender. Categorical variables were analyzed by calculating frequency and percentages. Assessment of immunomarker result was made and their association with the histological grade, with the help of Chi square test. A P value was set at < 0.05.

RESULTS

The results are made on one hundred cases of OSCC of different histological grades. There were fifty (53%) males and forty seven (47%) females, with a male to female ratio of 1.12:1. Mean age was 57.11 years with SD ±14.6. Sixty eight (68%) were WD-OSCC, with twenty one (21%) were MD-OSCC and eleven (11%) were PD-OSCC. The tongue was found in fifty seven cases (57%), followed by buccal mucosa thirty three cases (33%), lower lip six cases (6%), lower jaw two cases (2%), maxillary gingiva one case (1%) and hard palate one case (1%). WT1 was found weak or absent in the basal layer and in the infiltrating nest of cells. IHC of WT1 in OSCC with the percentage of stained cells were investigated in all of the histological grades of OSCC. (as sown in table 1).

Thirty sevent percent (37 cases) of the cells showed 0 reactivity, thirty seven percent (37 cases) showed 1+ reactivity, twenty two percent (22 cases) showed 2+ reactivity, four percent % (4 cases) showed 3+ reactivity. Seventy nine percent (54 cases) of positivity in WD-OSCC (68 cases), Nineteen percent (4 cases) positivity in MD-OSCC (21 cases) and forty five percent (5 cases) positivity in PD-OSCC (11 cases).

WT1 expression was detected more in nuclei of cells, however few of the cells showed cytoplasmic expression.WT1 was found weak or absent in the basal layer and in the infiltrating nest of cells. IHC (Immunohistochemistry) of WT1 in OSCC with the nuclear intensity of stained cells as investigated in all of the histological differentiation grades of squamous cell carcinoma (Table no.2).

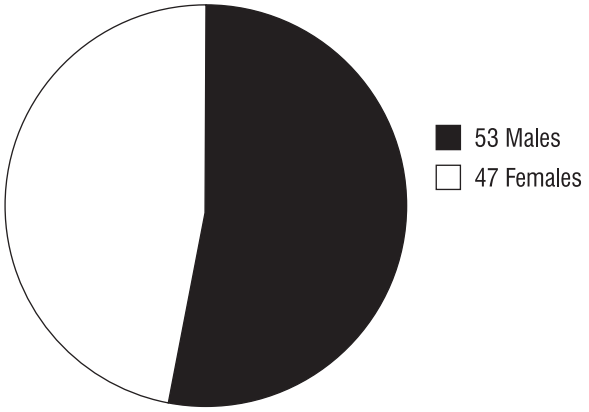

Fig 1: Gender distribution

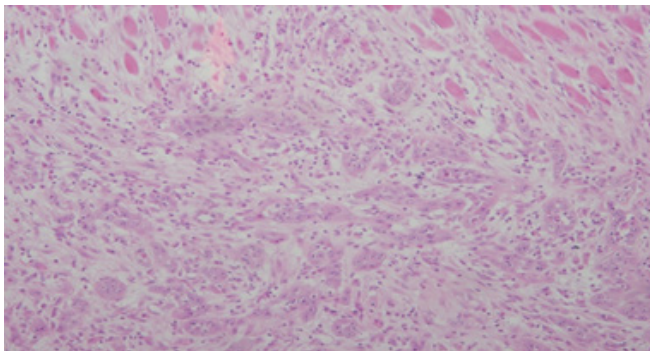

Fig 2: H/E staining of PD-OSCC at 10 X power magnification

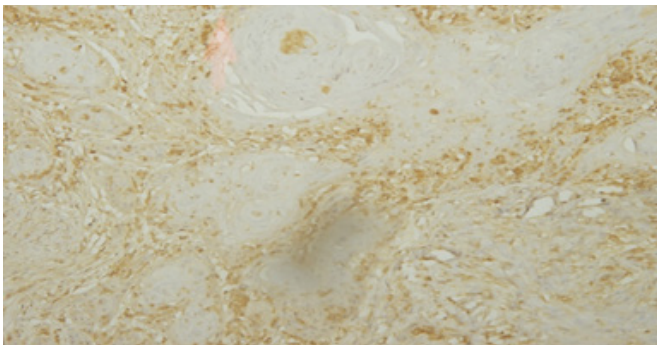

Fig 3: Immunoexpression of WT1 in PD-OSCC section showing densely nuclear stained tumor cells.

TABLE 1: WT1 EXPRESSION AND THE TUMOR SITE

Tumor Site	WT1 Expression	
	Positive	Negative
Tongue	34	23
Buccal Mucosa	23	10
Lip	03	03
Palate	01	0
Maxillary Gingiva	01	0
Lower Jaw	01	01

TABLE 2: WT1 EXPRESSION AND TUMOR DIFFERENTIATION

Tumor Differentiation	(n)	Wt1 Expression	
		Positive	Negative
WD-OSCC	68	54	14
MD-OSCC	21	4	17
PD-OSCC	11	5	6

TABLE 3: INTENSITY OF WT1 IN WD-OSCC

Intensity			
0+	1+	2+	3+
14	31	19	4

TABLE 4: INTENSITY OF WT1 IN MD-OSCC

Intensity			
0+	1+	2+	3+
17	03	01	-

TABLE 5: INTENSITY OF WT1 IN PD-OSCC

Intensity			
0+	1+	2+	3+
06	03	02	-

TABLE 6: WT1 NUCLEAR INTENSITY IN TUMOR DIFFERENTIATION OF OSCC

Tumor Differentiation	WT1 Intensity		P Value
	Low	High	
WD – OSCC	45	23	0.024*
MD – OSCC	20	01	
PD – OSCC	09	02	

*Significant

WD-OSCC

Fifty-four cases showed positivity out of sixty eight cases of WD-OSCC, 14 cases (20.5%) showed 0 reactivity, 31 cases (45.5%) 1+ nuclear reactivity, 19 cases (27.9%) showed 2+ moderate intentisity and 4 cases (5.8%) 3+ intensity (Table 3).

MD-OSCC

Four cases (19%) showed positivity out of 21 cases of MD-OSCC, 17 cases (80.9%) showed 0 reactivity, 3 cases (14.2%) showed 1+ nuclear reactivity, 1 case (4.7%) showed 2+ moderate intensity. (Table 4)

PD-OSCC

Five cases (45.4%) showed the positivity out of 11 cases of PD-OSCC, 6 cases (54.5%) showed 0 reactivity, 3 cases (18%) showed 1+ nuclear reactivity, 2 cases (18.18%) (Figure 2 and 3) showed 2+ moderate intensity (Table 5). The expression pattern was organized into two groups; high expression (2+ to 3+) and low expres-

sion (0 to 1+) and their relationship was correlated with histological grade of differentiation (Table.6).

DISCUSSION

Wilm's tumor 1 (WT1) is considered as a pleiotropic transcription factor and defined as tumor suppressor gene. The association of the WT1 in OSCC and its role in tumorigenesis is not yet established. A study on esophageal cancers by Oji et al.[7] showed positivity in all of the esophageal dysplastic differentiation and concluded the role of WT1 in tumorigenesis. In other study, Niksic et al.[13] found that the WT1 protein shuttled between the nucleus and cytoplasm and that a significant proportion of cytoplasmic WT1 protein was in association with ribonucleoprotein particles (RNPs) in mouse mesonephros-derived M15 cells, suggesting the involvement of WT1 in RNA metabolism. Our study showed the same phenomenon with positivity of WT1 in each of the histological grades of OSCC suggesting its role in tumorigenesis. WT1 was detected by nuclear staining in the cancer nests proliferating from the basal cell layer infiltrating the underlying connective tissue and absent to weak staining was found in the basal cell layer. Few of the cells also showed weak positivity in the cytoplasm suggesting its role as a transcription factor. The WT1 protein expressed in the cytoplasm of OSCC cells might be involved in RNA metabolism and play an important role in these cells. In this study, twenty three cases showed high nuclear staining with nineteen cases showing moderate intensity and 4 cases high intensity, embarking its role in dysplastic differentiation and providing an evidence of its role in tumor cells proliferation (P=0.024). Previous studies by Mikami et al. and Fattahi et al.[14], the nuclear and cytoplasmic staining of WT1 protein overexpression was observed more in WD-OSCC. Similarly, in our study WT1 protein was overexpressed in WD-OSCC. WT1 immunoexpression was also observed in PD-OSCC which concided with the studies of Oji et al.[7]

The role of WT1 in OSCC has not been studied extensively. No study has been carried out in Pakistan to the best of our knowledge. More studies are needed so that the prognostic significance of WT1 can be further studied. The relationship of WT1 with clinico-pathological parameters and the demographic factors needs to be researched for future conclusive studies. Previous studies were done at the genetic level and our study is confined to the WT1 protein. However, at the protein level, the cytoplasmic and nuclear staining suggests the WT1 tumorigenesis role. Multi-center studies with larger sample size are needed

CONCLUSION

The nuclear staining suggested the role of WT1 in cancer nests proliferation suggesting its oncogenic role in oral squamous cell carcinoma.

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5 Aamir Rafiq:	Literature review, data analysis.
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