ORAL SQUAMOUS PAPILLOMA AND SQUAMOUS CELL CARCINOMA;
DIAGNOSTIC UTILITY OF P53 IN DIFFERENTIATING

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ABSTRACT… Objectives: The objective of this study was to determine the diagnostic utility of p53 in differentiating oral squamous papilloma and squamous cell carcinoma. **Study Design:** A cross-sectional study. **Setting:** Pathology Department of Shaukat Khanum Memorial Cancer Hospital and Research Center Lahore. **Period:** Six months (6-10-10 to 6-04-11). **Patients and Methods:** A total of 100 oral biopsies fulfilling the inclusion criteria were collected. The expression of P53 was observed in biopsies of squamous papilloma and squamous cell carcinoma. Data was entered and analyzed on SPSS version 20 and was analyzed in same package. Sensitivity, specificity, positive predictive and negative predictive values were calculated along their confidence intervals at 95% level. **Results:** One hundred oral lesions were studied, of which 90 cases were of squamous cell carcinoma and 10 cases were of squamous papilloma. Majority of the patients were male (60%). Mean age was 46 years. P53 IHC stain had sensitivity of 92% and specificity of 80% and diagnostic utility of 91% in lesions of squamous cell carcinoma. **Conclusion:** P53 is positively expressed in oral squamous cell carcinoma and is negative in squamous papilloma. Hence evaluation of p53 expression can be a useful adjunct in the diagnosis of squamous cell carcinoma, especially in cases where it is difficult to distinguish between these two entities on morphological grounds.

Key words: Squamous papilloma, Squamous cell carcinoma, P53 Immunohistochemical stain.

INTRODUCTION

Oral cavity is affected by diverse type of lesions. These lesions are broadly divided into benign and malignant lesions.[1] Benign lesions account for 3% of all oral lesions while annual incidence of oral cancer in United States is 5 per 100,000 making it the 8th common cancer in males and 15th in females.[2] At Shaukat Khanum Memorial Cancer Hospital And Research Centre, a total number of 2,258 cases of oral cancer were reported over a period of 14 years, making it the 4th most common cancer as per their tumor registry.[3]

The most common benign oral lesion is squamous papilloma while the commonest cancer of oral cavity is squamous cell carcinoma (90%).[2] Tobacco smoking, oral snuff, alcohol intake, betel quid chewing, radiation, iron deficiency and oncogenic viruses are main etiological factors causing oral lesion.[1] Sometimes oral lesions present in such a manner that it often becomes difficult

for the pathologists to distinguish between squamous papilloma and squamous cell carcinoma solely on the morphological grounds and require immunohistochemistry. In such cases immunohistochemical (IHC) stain P53 is helpful.

P53 is a tumor suppressor gene, located at chromosome 17p13.1. Normal p53 protein (wild type) has a short half-life, so it is hard to detect in normal tissues. However, the protein can remain in the tissues longer due to mutations, or due to defect in the degradation pathway or by binding to other proteins.[4] Mutation of the *p53* gene has been reported in an average of 50% of human cancers.[5] Mutation and overexpression of p53 is common in head and neck cancers. The *p53* mutation has been reported in 8 cell lines and 2 xenografts of head and neck cancers[6.] Expression of p53 has been reported in 60% of laryngeal carcinomas[6], 37% of hypopharyngeal carcinomas[7], 45% to 55% of oral cavity cancers[8-10] and 27% to

61% of tongue carcinomas.[11-14] in premalignant oral dysplasia or carcinoma in situ, 46% of the lesions had mutations and 20% had overexpression of p53.[15, 16]

Abnormal p53 protein can be detected by P53 immunohistochemical stain (IHC). P53 IHC stain is considered positive when it shows brown nuclear staining. Molecular genetics of squamous cell carcinoma shows gene mutation and p53 is the most frequently altered gene (75%) while squamous papilloma does not show P53 mutations.[17-19] Study done by Melhem et al shows 91% sensitivity and 55% specificity of p53 expression in head and neck squamous cell carcinomas.[20] The aim of this study is to compare the expression of p53 in oral squamous papilloma and squamous cell carcinoma in order to determine whether the pattern of P53 expression can distinguish between these oral lesions and to determine its significance as a diagnostic tumor marker and if proved then p53 can be used to differentiate the cases which are difficult to diagnose on clinical and morphological basis. Hence can help the pathologist in early diagnosis of oral lesions.

MATERIALS AND METHOD
Study Design
Cross sectional case survey

Setting
The study was conducted in the Pathology department of Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH).

Duration of Study
The study duration was for six months

Sampling
Non probability purposive sampling

Sample Size
The calculated sample size was 100 cases, with 95% confidence level, 18.5% margin of error and taking expected percentage of oral lesions i.e 70.6% and taking sensitivity and specificity of p53 for malignant cases i.e 91% and 55% respectively study was performed on 55 cases.

Inclusion Criteria
1. Oral biopsy (incisional and excisional, clinically presenting as mass lesions).
2. Gender: Male and female
3. Age: More than 10 yrs.

Exclusion Criteria
1. Poorly preserved and poorly fixed specimens.
2. Previously treated patients (chemo/radiotherapy)

Methodology
One hundred cases of oral biopsies fulfilling the inclusion criteria were included in this study. These cases were referred to us from the surgical departments of various hospitals. These surgical oral biopsies were grossed, fixed in 10% buffered neutral formalin. Sections were processed and stained with hematoxylin and eosin. The immunohistochemical stain p53 was performed by deparaffenizing in xylene followed by rehydration. The sections were treated with hydrogen peroxide (3%) for 10 minutes at room temperature and washed in distilled water (D/W). They are then treated with microwave heating for 15 minutes in a citrate buffer (pH 6.0) for antigen retrieval, washed in D/W and treated with phosphate buffered saline (PBS) for 5 minutes twice. The immunohistochemical stains were carried out using DAKO AUTOSTAINER. The pathologist assessed the staining of p53 as positive (Squamous cell carcinoma) or negative (Squamous papilloma) as per operational definitions. The histopathological evaluation of these cases was subsequently carried out to confirm squamous cell carcinoma or squamous papilloma. These results along with the patient data including age and sex were recorded on the proforma.

Staining Interpretation and Reporting Criteria
The staining results of the tumor were expressed as the product of brown nuclear staining and percentage of positive cells. The whole section was scanned at low power in order to assess the general level of intensity throughout. The percentage of tumor cells was scored as follows:
>5%= positive staining of p53
<5%= negative staining of p53

Statistical Analysis

The collected information was entered into the SPSS version 20 and was analyzed accordingly. The quantitative variables that were analyzed include age. Mean and standard deviation was calculated for quantitative data. The qualitative variables that were analyzed include sex. Frequency of percentage was calculated for the qualitative data. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic utility of p53 expression for the diagnosis of squamous cell carcinoma were calculated by generating 2 x 2 tables, taking histopathology as gold standard.

RESULTS

A total of 100 cases were studied during the study period of 6 months from 06-10-2010 to 06-04-2011. The age ranged between 20 years (minimum age) and 88 years (maximum age). The mean age of patients was 46 ± 10 years. Out of 100 cases, 60 patients (60%) were male and 40 patients (40%) were female. A histologic diagnosis of Squamous cell carcinoma was made in 90 cases while p53 positivity was seen in 83 cases. P53 showed a sensitivity of 92.22%, specificity of 80%, diagnostic utility / accuracy of 91%, positive predictive value of 97.65% and negative predictive value of 53.3%.

P53	Histopathology (Gold Standard)		Total
	Squamous cell carcinoma	Squamous papilloma	
Yes (positive staining)	83	2	85
No (Negative staining)	7	8	15
Total	90	10	100

Table-I. Comparison of p53 Staining versus Histopathology

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	92.22%	(84.81, 96.18)
Specificity	80%	(49.02, 94.33)
Positive Predictive Value	97.65%	(91.82, 99.35)
Negative Predictive Value	53.33%	(30.12, 75.19)
Diagnostic Accuracy	91%	(83.77, 95.19)

DISCUSSION

Oral cancer is one of the commonest cancers and constitutes a major health problem in developing countries, representing the leading cause of death.[21] Epidemiological studies have shown that the site of occurrence for oral cancer differs widely. Tongue, lip and floor of mouth are the most frequent sites of lesions of squamous cell carcinoma in the oral cavity.[22] In our study excluding the cases in which site was not specified, the tongue is the most commonest site of lesion. Mean age of patients with squamous cell carcinoma in this study is 46years. There is a slight male predominance in our study. P53 immunohistochemical stain was done and was positive in 83 out of histologically diagnosed 90 cases of squamous cell carcinoma with a sensitivity of 92% and specificity of 80%. False negativity of p53 was seen in 7 cases of squamous cell carcinoma. P53 immunohistochemical stain was negative in 10 cases diagnosed as squamous papilloma.

Squamous cell carcinoma is the most frequently occurring tumor of oral cavity.[23] Malignant tumors result from accumulation of genetic alterations, which lead to overexpression of oncogenes and inactivation of tumor suppressor genes. The consequences of these changes in cells progressing to malignancy are the loss of negative regulation of cellular proliferation in normal programmed cellular death mechanisms (apoptosis). p53 belongs to this family of genes.

P53 is the the tumor suppressor gene located on short arm (p) of chromosome 17. Mutations of the p53 gene are the most common genetic alterations associated with human cancer. An important consequence of these various mutations is post-translational stabilization of the altered p53 protein, leading to elevated p53 levels in tumor cells, which can be detected by p53 IHC stain. It has been well documented that alterations of levels of p53 expression are early events in the development of carcinoma of oral cavity. Expressions of p53 has been found in dysplastic oral epithelial cells.[22] There is no doubt that p53 expression is an important early step in the development of carcinoma of oral cavity.

Kannan et al conducted a study to see p53 expression in normal mucosa, dysplastic and malignant lesions of the oral mucosa.[23] Overexpression of p53 was seen in 67% of oral squamous cell carcinoma (OSCC).[23] In a Pakistani study by Panjwani and Sadiq, p53 immunoexpression was positive in 75% of oral epithelial diseases (OED) and 76.8% of squamous cell carcinoma (SCC).[24] Melhem et al conducted a study which yielded that p53 IHC stain expression has 91% sensitivity and 55% specificity in oral squamous cell carcinoma which is comparable to our study.[25] P53 showed a sensitivity of 92.22%, specificity of 80%, diagnostic utility /accuracy of 91%, positive predictive value of 97.65% and negative predictive value of 53.3%. Expression of p53 will also help the oncologists to assess the prognosis and effectiveness of their chemotherapy because tumors with overexpression of p53 have worse prognosis.

CONCLUSION

This study indicates that p53 is positively expressed in oral squamous cell carcinoma and is negative in squamous papilloma. Hence evaluation of p53 expression can be a useful adjunct in the diagnosis of squamous cell carcinoma, especially in cases where it is difficult to distinguish between these two entities on morphological grounds.
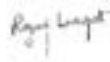
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AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Dr. Najiya Barkat	Data collection	
3	Asif Hanif	Statistical analysis	