ORIGINAL ARTICLE

Immune-Expression of p53 in Oral Preneoplastic and Neoplastic Squamous Lesions

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ABSTRACT

Objective: Oral cancer is a major problem and accounts for about 5% of all cancers. The incidence is however higher in South Asia. Besides tobacco and areca nut chewing, oral oncogenesis is associated with Human Papilloma Virus (HPV). Oral squamous carcinoma usually develops through the stage of dysplasia. Alteration in p53 expression is frequently observed in oral carcinogenesis and is also linked to the evolution of preneoplastic lesions to infiltrating cancers. This study aimed to detect p53 immuno-expression in oral preneoplastic and neoplastic squamous lesions and to evaluate the correlation between these lesions and p53.

Methodology: Hundred cases of Oral Squamous Cell Carcinoma (OSCC) and 50 cases of Oral Premalignant Lesions (OPL) were included. Selected tissues were stained with Hemotoxylin & Eosin (H&E), followed by immunohistochemistry for p53.

Results: Nearly half (48%) of OSCC were well differentiated, 37% moderately, and 15% were poorly differentiated. Majority (74%) of the presented cases were in advanced stages. In OPL cases, 50% presented as mild dysplasia, 32% as moderate, and 18 % as severe dysplasia. p53 nuclear staining was positive in 70 (70 %) cases of OSCC and 27 (54 %) of OPL. Correlation of p53 with tumor histological grade was significant (P<0.05).

Conclusion: High frequency and strong positivity of p53 tumor suppressor gene in oral dysplasia and OSCC have been implicated as an early indicator in oral carcinogenesis. Higher frequency of p53 positivity in carcinoma than dysplasia can be considered as a predictive marker. P53 may serve as a potential target for individualized therapy.

Key words: Oral Squamous cell carcinoma, Oral premalignant lesions, p53

عنوان: منہ کے کینسر سے پہلے اور کینسر ہونے کے بعد P53 کی مدافعتی اثرات پر تحقیق۔

مقصد: منہ کا کینسر دنیا بھر میں ایک سنگین مسئلہ ہے پائے جانے والے تمام اقسام کے کینسر میں منہ کے کینسر 5% پائے جاتے ہیں۔ جبکہ جنوبی ایشیا میں اس کی تعداد کافی زیادہ ہے۔ تمباکو نوشی اور چھالیہ چبانے کے ساتھ ساتھ منہ کے کینسر کی ایک وجہ ایک خاص قسم کا وائرس HPV بھی ہے۔ اس مطالعہ کا مقصد منہ کے کینسر سے پہلے اور کینسر ہونے کے بعد زخموں پر P53 کی مدافعتی اثرات اور ان کے باہمی تعلق کی جانچ کرنا ہے۔

طریقہ: اس مطالعہ میں OSCC کے 100 اور منہ کینسر کی وجہ سے چھوٹے زخموں سے متاثر 50 مریضوں کو شامل کیا گیا۔ منتخب کردہ بافتوں کو H&E سے نشاندہی کے لیے داغ دار کیا گیا۔ اور P53 کے مدافعتی اثرات کو جانچا گیا حاصل ہونے والے نتائج کو SPSS کے ورژن 16 پر جانچا گیا۔

نتائج: حاصل ہونے والے نتائج میں OSCC میں P53 کی وجہ سے تقریباً آدھے (48%) مریضوں میں اچھا، 37 % درمیانی فرق پایا گیا اور 15% میں اس کا کوئی خاص فرق نہیں پایا گیا۔ اکثریت %74 ایڈوانس اسٹیج پر تھے۔ OPL میں %50 مریضوں میں ہلکا، 32% فیصد میں معتدل اور 18% فیصد مریضوں میں انتہائی درجہ کا dysplasia پایا گیا۔ مشاہدات سے پتہ چلا کہ OSCC کے 70% فیصد اور OPL کے 27(54%) مریضوں میں نیوکلیائی اسٹیننگ کے مثبت اثرات پائے گئے۔ کینسر کے گزشتہ درجات اور P53 کے اثرات کے درمیان خاص تعلق پایا گیا۔

حاصل مطالعہ: حاصل ہونے والے نتائج سے معلوم ہوا کہ OSCC اور OPL کی جلد تشخیص ہونے میں P53 کی مثبت موجودگی مدد کر سکتی ہے۔ P53 کی موجودگی کارسنوما کی جلدی تشخیص میں مددگار ہو سکتی ہے۔

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INTRODUCTION

Carcinoma of oral cavity ranks as the 8th most common malignancy worldwide and accounts for about 5% of all cancers. Near about 275,000 new cases of oral cancers and 128,000 deaths are recorded worldwide annually[1,2]. Incidence is higher in South Asian countries like India, Pakistan, and Sri Lanka, and some European and Latin American regions[3]. An American study estimated 49,670 new cases and approximately 9,700 deaths occurred in US in 2012[4]. Oral cancer is a leading malignancy of head and neck region in India and shares approximately one third of the worldwide cancer burden[5]. The annual cancer registry of Shaukat Khanum Memorial Hospital reported 292 (4.8%) new cases of oral cavity cancers in 2016 and ranked it as the 6th amongst all reported malignancies of adults in Pakistan[6].

In our part of the world, the major risk factors for oral cancer, include tobacco and areca nut chewing. More than 600 million of world population is expected to have tobacco chewing habits and majority population is presumed to belong from South Asia[7]. Other known factors include high risk type human papilloma virus (HR-HPV) i.e. HPV16 and 18, poor hygiene and genetic predispositions[8].

Oral premalignant lesions (OPL) or dysplasia are abnormal lesions which may progress to invasive cancers and may also outrun the subsistence of oral squamous cell carcinoma (OSCC)[9]. As per 2005 WHO classification; oral dysplasia is classified according to the cellular morphology and architecture into three grades; mild, moderate and severe dysplasia[10]. Tumor grade is the extent of differentiation and microscopic architectural organization of lesion. It indicates tumor aggressiveness in context of its rate of growth and spread. A number of grading systems have been developed which are modifications of the initial Broder's system[11]. Most commonly used system is WHO system that depends on the cytological characteristics and architectural organization. The system classifies squamous cell carcinoma into four increasing grades based on the degree of differentiation i.e., Well, Moderate, Poor, or Undifferentiated respectively[12].

Four progressive clinical stages of OSCC from stage I to IV are described in the commonly used staging system TNM, based on tumor size and metastasis[13]. p53 protein acts as a master transcriptor gene[14]. p53 possesses tumor suppressor role and repairs DNA in case of any damage. Normally, p53 is short-lived and cannot be observed with immunochemistry, while mutant p53 is more firm and shows positivity by immuno-staining. p53 expression is very well appreciated during active cell turn over and this positive staining may correlate with genetic alterations. These inactivation mutations allow oncogenetic factors to predominate; directing the pathway towards malignancy[15]. Dysregulation of p53 pathway is a general mechanism for tumorogenesis and is a commonly occurring genetic alteration incorporating DNA repair, apoptosis, and angiogenesis[16,17]. Nearly all human cancers possess p53 alteration and more than 80% are point mutations[18]. Strong expression of p53 is clearly observed at the invasive front of OSCC and it also corresponds to the tumor morphology, worse prognosis, and recurrence[19].

In recent years, OSCC has gained attention due to rising prevalence, failure in regression, and its recurrence. Initiation and evolution of OPL to infiltrating cancers is linked to p53 dysregulation; therefore, detection of p53 in dysplasia may be considered as a potential marker for progression to frank malignancy.

The current study aims at detection of the over expression p53 in preneoplastic and neoplastic squamous lesions of oral cavity and to establish an association between p53 and histological grades of these lesions.

METHODOLOGY

This study was a cross sectional analytical study and was approved by the Institutional Review Board at Dow University of Health Sciences. It included clinically diagnosed, histopathologically proven one hundred (100) cases of OSCC and fifty (50) cases of OPL received from Otolaryngology ward of Civil Hospital, Karachi and DIKIOHS and DUHS from Jan 2012 to Dec 2014.

Relevant clinical data was recorded on a designed proforma. All recently diagnosed cases of OPL and OSCC were collected from Otolaryngology ward of Civil Hospital, Karachi and Dr. Ishrat ul Ebad Khan Institute of Oral Health Sciences (DIKIOHS) at DUHS. Selected cases were biopsied and the cases with representative pathologies were included, while all inadequate biopsies, and patients receiving chemotherapy / radiotherapy were excluded from this study. OPL with invasive foci were considered as carcinoma.

Tissues obtained were fixed and subjected to routine processing and staining with Hemotoxylin & Eosin (H&E). Sections of OPL were classified histologically into mild, moderate, and severe dysplasia by Barnes classification and OSCC were graded into well, moderately and poorly differentiated according to WHO classification[10,12].

For immunohistochemistry 4µm thick tissue sections, mounted on poly-L-Lysine coated slides were heated at 65°C for 1 hour, followed by deparaffinizing / hydration, with xylene and different dilutions of ethanol (100%, 95%, 70%, 50%, and 30%). Antigen retrieval was done in 500ml dH2O/ Citrate buffer solution in pressure cooker for 20 minutes for 9 minutes followed by cooling down to room temperature within 10-20 minutes. Endogenous peroxidase was inactivated. Incubation was done with Mouse monoclonal anti p53 Clone DO7 (cat: no. 453M-96; Cell Marque) diluted in diluent per manufacturer's recommendations. Primary antibody was applied to each section and incubated overnight in humidified chamber (4 ℃) followed by washing with TBST. Secondary HRP-conjugated anti-rabbit antibody applied to each section; incubated for 30 min at room temperature and washed with TBST.DAB was added for 01 minute; followed by counterstaining with Harris' Hemotoxylin and mounting. One positive control (breast carcinoma) and one negative were run with each batch. For negative control primary antibody was excluded. Sections were observed at 10x, 20x and 40x for immunohisto-chemical evaluation.

Results were analyzed in semi-quantitative manner by multiplying intensity and percentage of cells stained. Intensity was taken as 1 for mild, 2 for moderate and 3 for marked. Percentages of stained cells was graded as 1 (1-10%), 2 (11-50%), 3 (51-80%) and 4 (81-100%) staining of cells. Final score of 0-12 was obtained by multiplying the two variables. A score of 4 or above was considered positive.

Statistical analysis was done by SPSS 16. Univariate analysis using Pearson's Chi Square test was applied to assess correlation between dependent and independent variables. P-value of <0.05 was taken as significant.

RESULTS

Among OSCC, 48 (48%) were well differentiated, 37 (37%) moderately and 15 (15%) were poorly differentiated. Majority patients presented with stage IV (39%), followed by stage III (35%), stage II (20 %) and stage I (6 %) respectively.

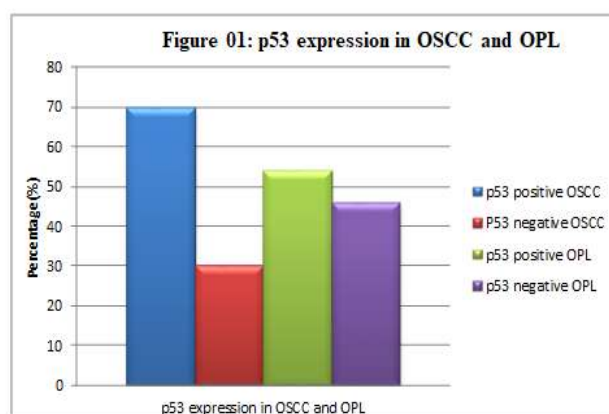Considering OPL, 25 (50 %) showed mild, 16 (32%) moderate and 9 (18%) severe dysplasia respectively.

Figure: 1: Most of OSCC as well as OPL cases showed positive p53 immuno- staining

Table 1: Association of p53 Immune-Expression with Staging and Grading of Oscc and OPL Grading

	p53		Total	P-Value
	Negative (70%)	Positive (30%)		
TNM Staging				
I	3(50)	3 (50)	6	
II	6(30)	14(70)	20	0.532*
III	8 (22.9)	27(77.1)	35	
IV	13(33.3)	26 (66.7)	39	
Grading				
Well Differentiated	13 (27.1)	35(72.9)	48	
Moderately differentiated	7 (18.9)	30(81.1)	37	0.003**
Poorly differentiated	10 (66.7)	5 (33.3)	15	
OPL Grades	Negative 23 (46%)	Positive 27 (54%)		
Mild	12 (48)	13(52)	25	
Moderate	7 (18.9)	9 (56.3)	16	0.960~
Severe	4 (44.4)	5 (55.6)	9	

*Significant at 0.05
**Significant at 0.01
~Chi-sq Test P-value with More than 20% Cells Proportion
Test: Chi-square for Association, `Fisher Exact Test

A significant relationship (p<0.05) was found with tumor grade and p53 staining. P53 nuclear staining was positive in 72% of well differentiated, 81 % of moderately and 33% of poorly differentiated tumors. No significant relation was found between p53 with tumor stage. Relationship between p53 and OPL was also not significant. (Table 1)

DISCUSSION

Oral cancer is a problem of enormous magnitude for Pakistan due to high morbidity and mortality. This study showed the maximum number of cases in advanced stages III and IV (35% to 39%) and 74% collectively. This is substantiated by Li et al and Agrawal et al reporting 65% and 72% cases with stages III and IV respectively[20,21].

However, some studies from developed countries document, more cases with primary stages (I and II); 86.66% and 64.3%, and less number of patients with advance malignancy (stage III and IV); 13.3% and 35.7% respectively[22,23].

Pakistan having a low literacy rate with limited health resources and awareness regarding the magnitude of OSCC, majority of the patients here present in later stages due to social and self-impediments. Varying degree of architectural variations in premalignant and malignant oral lesions was observed in this study. Well differentiated morphology was frequent, followed by moderately and poorly differentiated histologies. Other studies on same ground support our findings of well differentiated morphology being common, while poorly differentiated being the least common[20-24].

Dysregulation of tumor suppressor gene p53 is common in carcinogenesis. Oral cancers also belong to similar group of cancers with noticeable p53 alterations. Present study reveals, frequency of p53 alterations to be quite high (70 %) in OSCC, which is comparable to most previous studies in the world[24,25]. Conversely, studies from Rothenberg et al and Perez et al have shown a much lower frequency of p53 mutation[26,27]. these disparities in statistics are quite possible due to variations in demographic and risk factors.

A considerable variation was observed in immune expression of p53 with tumour grade and stage. We could not find parallel relationship between tumour stage and p53 similar to Abrahao et al and Bansal et al, who also failed to establish a relationship between p53 dysregulation with advancing stages of malignancy[25,28]. In contrast, a few studies show a significant relation between p53 and tumour size[20,29].

The possible explanation for this variability may be due to other prevalent risk factors or accumulation of multiple mutated genes with cancer stage progression. Our study established a strong correlation between p53 mutation and histological grade of OSCC. Increased expression from low grade carcinoma to higher grades malignancy was consistent with the findings of other studies worldwide.[20,29] This over expression of p53 determines high cell turn over and aggressive behavior of tumour.

Our study revealed progressive expression of p53 from mild to severe dysplasia. Similarly, other studies have shown progressive expression of p53 with severity of OPL[30,31]. However, p53 staining in OPL was insignificant. Genetic alterations involving p53 is a frequent event in invasive cancers than intraepithelial lesions. The discrepancy could be due to switching of oncogenetic pathways and genetic aberrations other than p53.

This study identified distinct immune-expression of p53 in series of oral pathologies ranging from dysplasia to high grade OSCC, in Pakistan. Active expression of p53 indicates cell proliferation, aggressiveness and lethal outcomes of disease and may predict chances of recurrence. In this concern detection of p53 in dysplasia and early carcinoma in target population may facilitate the selection of individualized specific therapy with fewer side effects, better survival rates and prevention of recurrence.

The major limitations were financial constraints and difficulty in obtaining material for OPL; since tissues obtained were far fewer than of OSCC and OPL are usually not biopsied until having strong suspicion for malignancy. Moreover, a considerable number of clinically diagnosed OPL revealed invasion.

CONCLUSION

This study showed strong expression of p53in oral dysplasia and OSCC which were also implicated as an early indicator in oral carcinogenesis. Therefore p53 positivity can be considered as a predictive tumor marker, in that way helping in selection of targeted therapy.

Oral cancer is a matter of great concern in Pakistan. It is highly recommended that further extensive researches including larger sample size and investigating alterations in other genetic pathways should be carried out. Moreover, molecular studies for detection of events in oral carcinogenesis with the provision of possible therapeutic options and prognosis should be considered. Due to rise in the incidence of oral premalignant and

malignant lesions, mass screening programmes and awareness campaigns should be raised at the government level.

Conflict of interest: The authors declare no conflict of interest.

Authors' contribution: Dr Saadia Akram conceived the study, and worked on data collection, analysis, and write up. Dr Saba Sattar participated in literature search, and worked on introduction, discussion and write up. Dr M. Amir Mirza contributed in data collection and analysis. Dr Talat Mirza reviewed and edited the article. Dr Sarah Mirza participated in data collection and analysis. All authors contributed to the final manuscript.

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