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ORIGINAL ARTICLE

Association of P53 expression with clinicopathological gradings of oral squamous cell carcinoma patients in Hyderabad Sindh.

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ABSTRACT… Objective: To evaluate the frequency and association of p53 gene with increase histopathology categorizing of "oral squamous cell carcinoma" patients. **Study Design:** Comparison Cross-sectional Research. **Setting:** Isra Dental College Hyderabad. **Period:** 1 November 2021 to 31 October 2022. **Material & Methods:** Sixty patients of identified oral squamous cell carcinomas were chosen by consecutive sampling from patients reporting at Oral surgery department Isra dental college, Poly-L-lysine solutions was used over all of the formalin-fixed, paraffin embedded segments, and was prepared for immunostaining. Primary antibody and counter stain hematoxylin was used and dehydrated through graded alcohol dilutions. Sections were mount by Dpx and results interpreted. **Results:** Sixty instances of oral squamous cells carcinoma divided into histological grading well differentiated OSCC, moderately differentiated OSCC, and poor differentiated OSCC based on their histological characteristics. Fifty-two (87%) of the participants in this study were male, while just eight (13%) were females. Association of p53 were found insignificant with age. Gender and other clinical factors of OSCC, the prevalence of p53 gene countenance was found increase in oral squamous cell carcinoma. But there was no any significant association found with the increase histological grading of OSCC. **Conclusion:** Prevalence of P53 gene expression found increase in OSCC. While P53 expression was not significantly found with increase histological grading of oral squamous cell carcinoma.

Key words: Histological Grading, Immunohistochemistry, P53, Squamous Cell Carcinoma.

INTRODUCTION

There has been a rise in the incidence rate and death due to Squamous Cell Carcinoma of the oral cavity, It is the sixth least collective cause of cancer death globally, however the third least frequent in developing countries.[1] Cancer reported the main or subordinate source of mortality for persons below the period of 70 years in 112 of 183 countries in recent years, Patients with precancerous lesions, including oral sub mucous fibrosis and Leukoplakia are the typical source of OSCC (OSF).[2] The two greatest major etiological aspects in the development of oral tumor are the use of tobacco products and the consumption of alcoholic beverages, both of which are on the rise.[3] Furthermore, it has recently been reported that a diet deficient in fresh fruits and vegetables, as well as exposure with high-risk human papillomavirus (HPV) genotype, have a character in the aetiopathogenesis of oral SCC.[4] Oral squamous cell carcinoma is very frequent in south Asian nations like India, Bangladesh, Pakistan, etc.[5]

The development of oral cancer involves a chain reaction of events that might cause cell cycle disruption at any stage.[6] In latest years, investigations have identified modifications (Mutations) of gene suppressing in the onset of carcinogenesis, with P53 being the most typically altered tumor suppressor.[7] On the short arm of chromosome 17, at the 13.1 genetic locus, 11 Exons make up the tumor suppressor p53.[8] Tp53 protects genome integrity and encrypts, a 393 "amino acid phosphoprotein" (protein p53) that controls the cell evolution sequence, repairs

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muted DNA, and inhibits cellular proliferation via mortality of injured cells, in response this gene will increase expression when a cancerous cell started to grow in the human body.[9-10] Furthermore, in tumors at certain sites, p53 status has demonstrated predictive value as a prognostic factor for the progression of the ailment and the existence of tumor patients.[11]

In Pakistan, carcinomas of the lip and oral cavity are the third most common type of malignant tumor.[12-13] The rising incidence of oral squamous cells carcinoma in the global population creates motility, making the diagnosis and treatment of this malignancy difficult for medical experts.[14] This research was conducted to assess the appearance of p53 (tumour suppressors gene invention) and the connection among different histological grades and p53 countenance in person with "Oral Squamous Cell Carcinoma" (OSSC).

MATERIAL & METHODS

This comparative research remained approved out at the Histopathology Department, Isra dental college, Faculty of dentistry and allied sciences, Hyderabad Pakistan, from 1st November 2021 to 31st October 2021. The Ethical Committee (IU/RR-10/AQK/2021/1951**)** of Isra University Hyderabad reviewed and approved the study protocol.

In this study total of sixty patients of both genders with ages 20 to 70 years clinically and histologically diagnosed to have oral squamous cell carcinoma were comprised in this research. Specimens were placed from the branch of oral surgery and processed in oral pathology lab, specimens were categorized on histological categorizations grade I, II, and III As according their degree of histological differentiations are classified as well-differentiated, poorly differentiated or moderately differentiated oral squamous cell carcinoma and Clinicopathological information including age, gender, habit including cigarette smoking, alcohol intake and betel nut chewing etc, medical history, locations of participants and histological rank was recorded of each participants.

Sixty (60) diagnose patients of oral squamous cell carcinoma were subjected to immunohistochemical analysis for detection of p53. 5μm thin layer of Paraffin embedded specimen that was formalin-fixed and covered with such a poly-L-lysine solution were used over the section. For the better results, the sections were placed at 56 °C for 24 hours after being put on coated slides. In order to deparaffinize (paraffin wax removing) the sections, xylol was used. The segments were then dehydrated in a series of progressively more diluted alcohols, ending with Tris buffer solution. Citrate buffer was used to retrieve antigens by boiling it to the point of one whistles in a cooking pot, then letting it drop to room temperature without lifting the lid. Sections were treated with 3% "hydrogen peroxide in methanol" for 10-15 minutes, at which point the indigenous peroxidase activity was inhibited.

Immunohistochemical staining was performed by Streptavidin Biotin Technique for detection of p53 gene. The specimen sections were supplemented with a "monoclonal antibody" (mouse monoclonal anti-p53) at room temperature and gestated for 24 hours at 38 °C in a humid atmosphere. The portions remained then cleaned 3 times for 10 minutes with TBS, reacted for 30-minutes at room heat in a humid chamber with biotinylated secondary (Link) antibodies, and rinsed three times with TBS. Sections were treated in streptavidin for 45 minutes at room temperatures in a humid environment before being rinsed in TBS and gestated in newly produced solution of "3,3' diaminobenzidine tetra hydrochloride" (DAB). This was made by reducing 1 drop of DAB chromogen in 1 ml of DAB substrates. The DAB substrate was formerly utilized to localize antibody mandatory Portions were cleaned in filtered liquid, counter stain with hemotoxylin for a maximum of three minutes, dehydrated via a series of ethanol concentrations, rinsed in xylol, and mounting with DPX. Control sample slides were treated with specific antibody, while trackball mouse blood was substituted for specific antibody in control treatment slides.

Screening of less than 10% of keratinocytes (-) discoloration of 10% to 33% of keratinocytes (+), and discoloration of more than 66% of

keratinocytes (+++) were the 4 types used to classify the number of p53-stained nuclei. A qualitative scale from 1+ (a distinct but light stain) to 2+ (a somewhat deeper stain) to 3+ (a very dark stain) was employed to categorize the stain's strength (3+).[15]

RESULTS

Fifty-two (87% of the total) men and eight women were included in the study of oral squamous cell carcinoma (OSCC). Mean age was greater for females than male (mean age of female = 43.21±21.5 years, while average age of male = 36.14±14.1 years). Among the patient's demographic details, 44 (73.3%) used smokeless tobacco, 8 (13.3%) did not use tobacco, and seven (11.6% smoked) (shown in Table-I and III).

Patients having "oral squamous cell carcinoma" have been additional categorized into seven groups, as indicated in Table-I, based on the extent of oral cavity involvement. The most prevalent area of infection was the oral epithelium (26 individuals), whereas the bottom of the tongue had the fewest instances. (only 1 enduring).

Total sixty samples were diagnosed after clinical and histological examination of patients, total 51 (85%) patients were diagnosed with well differentiated OSSC, 6 patients (10%) with moderately differentiated and 3 patients (5%) were diagnosed with poorly differentiated OSSC (Shown in Table-II).

Sixty OSCC tissue specimens were exposed to Immunohistochemical discoloration for appearance of p53 consuming the Streptavidin Biotin Technique. A total of 51(85%) samples showed positive immunoreactivity while 9 (15%) tissue samples showed negative reactivity as mentioned in Table-IV.

Prevalence of P53 appearance was increasing in oral squamous cell carcinoma but there was no any important correlation found with the histological grading. According to Table-V, the highest percentage of P53 manufacture was found in well-differentiated OSCC (41.7%), preceded by judiciously distinguished (36.7%), and poor-differentiated OSCC (6.7%).

Variables	n (%)
No Tobacco Use	08 (13.3)
Smokeless tobacco	44 (73.3)
Smoking	07 (11.6)
Alcohol Site of oral squamous cell carcinomas	01 (1.6)
Buccal mucosa	26 (43.3)
Tongue	5 (8.3)
Retromolar area	4 (6.6)
Floor of mouth	1 (1.6)
Palate	4 (6.6)
Alveolus	15 (25)
Lip	5 (8.3)
Male	52 (86.6)
Female	8 (13.4)
Age (years) [Mean±SD]	
Male	36.14 ±14.1
Female	43.21±21.5
Total	60 (99.9)

Table-I. Details study of population (n=60).

Histopathological Categorizing	Patients Frequency	% age of the Patients
Well Differentiated OSCC	51	85%
Moderately Differentiated OSCC	6	10%
Unwell (poorly) Differentiated OSCC	3	5%

Table-II. Oral squamous cell carcinoma; histopathological differentiation

Age Groups (years)			0.08
21-39.9		05	20
40-59.9		06	26
≥60		01	02
Gender			0.06
Age (Mean±SD)	Male	08	44
	Female	04	04
Total		12	48

Table-III. Relation between tissue p53 immunoreactivity and age, gender (p-value)

DISCUSSION

The research of the molecular pathogenesis of OSCC can aid in the research for microsatellite tools that may be capable of anticipating the clinical behavior of the tumour, which would be beneficial in regenerative medicine for OSCC.[16]

A) Level of p53 immunohistochemical stain	OSCC lesions
Absence or infrequent keratinocyte staining (-)	9 (15%)
Staining of 10-33% of keratinocytes (+)/ Supra basal staining in normal oral mucosa	27 (45%)
Staining of 33-66% of keratinocytes (++)	10 (16.7%)
Staining of superior than 66% of keratinocytes (+++)	14 (23.3%)
B) Staining intensity of p53	OSCC lesions
None	9 (15%)
Certain but light stain (1+)	24 (40%)
Dimmer stain (2+)	23 (38.3%)
Most intense stain (3+)	4 (6.7%)
C)Tissue p53 Immunoreactivity	OSCC lesions
Positive	51 (85%)
Negative	9 (15%)
Total	60 (100%)

Table-IV. P53 immunohistochemical staining level in tissue specimens of the study participants of "oral squamous cell carcinoma"

Histopathological Features	Tissue p53 immunoreactivity		Total	P-Value (Chi-square Test)
	Negative	Positive		
Well Differentiated SCC	6(5%)	45(41.7%)	51(46.7%)	0.683*
Moderately Differentiated SCC	2(8.3%)	4(36.7%)	6(45%)	
Poorly Differentiated SCC	1(1.7%)	2(6.7%)	3(8.3%)	

Table-V. Association of p53 with histological grading of oral squamous cell carcinoma

The present study reported that cigarette-smoking people are at high risk of oral squamous cell carcinoma followed by alcohol and betel nut chewers, current study reported that prevalence of males is high as compared to females. While there was not found an association with the age of the patients.[17]

Among OSCC lesions, a statistically inconsequential relation was perceived among tissue p53 immunoreactivity with age and gender. These findings are in obedience to the observations reported by Abbas Saleem Khan et al. However, a study done by Gatto et al., revealed a statistically important relation among p53 immunoreactivity, age, and gender of OSCC lesion.[18]

According to present study, p53 found an increase in oral squamous cell carcinoma in most of the participants, and the highest frequency of tissue p53 immunoreactivity was noted among well differentiated OSCC as compare to Poorly differentiated OSCC and moderately differentiated OSCC. There was not found any association of p53 appearance with increased histological grading of OSCC and results were not found insignificant.

Another study was conducted in Pakistan, according to Khan AS, at el study reported nonsignificance p53 expression association with histological gardens.[19]

Another study was conducted in Thailand, Kerdpon D at el (2001) the study found association of smoking and alcohol intake with OSCC but not any important association of p53 appearance with increase histological grading of OSCC.[20]

On other hand Indian researcher had reported significant association of p53 expression with increase histological gradings in his research "Pandya JA, at el (2019).

LIMITATIONS

Increase Sample size needed for more athenticity
The samples that did not express protein p53 on IHC and showed detection of mutations on molecular analysis.

CONCLUSION

Prevalence of P53 gene expression found

increase in OSCC. But P53 over expression was not significantly found with increase histological grading of oral squamous cell carcinoma (OSCC). **Copyright© 15 Feb, 2023.**

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Waqas Iqbal	Study design, Patient selection, data collection, experimental work and histopathological evaluation.	
2	Uzma Tariq	Study design, questionnaire design, Literature review.	
3	Shahzaman Memon	Data analysis, suggestions, data interpretation.	
4	Khushbu Lohana	Review and proofreading.	
5	Hafiz Mahmood	Data collection, drafting in literature work.	
6	Ayesha Iqbal	Drafting discussion chapter data analysis, Drafting the manuscript.	