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Original Research Article

Immunohistochemical Analysis of P53 Protein Expression in Oral Lesions

Kasapa Shyam Sunder

Associate Professor, Department of Pathology, RVM Institute of Medical Sciences and Research Center, Laxmakkapally, Mulugu, Siddipet District, Telangana

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Corresponding author: Dr. Kasapa Shyam Sunder

Conflict of interest: Nil

Abstract

Background: The oncoprotein p53 actively contributes to the initiation and/or development of oral carcinogenesis. It has been demonstrated that many human solid tumors of squamous origin, including oral squamous cell carcinomas, express mutant p53. The current study aimed to determine the expression of p53 in oral cavity lesions and its associations with tobacco use, including eating betel nuts with or without tobacco.

Methods: Tissue samples from cases of Cases that exhibit leukoplakia, erythroplakia, mucosal ulcer, and growth were obtained. A detailed history of smoking habits, betel nut chewing, and consumption of pan or gutkha, specifying the total duration, frequency per day, and the amount consumed daily. Three slices measuring 4-6 µ underwent immunohistochemical staining for p53 using autoimmunostainer (benchmark) to evaluate its expression. On IHC-stained sections, the analysis of p53-positive cells was carried out.

Results: Out of the lesions the SCC well differentiated showed the highest intensity. Similarly, 50% of cases of moderately differentiated showed moderate intensity and high-intensity staining. All the benign, premalignant, and inflammatory lesions were found to be negative or weakly positive for p53 staining. The histopathological diagnosis most common lesion was squamous cell carcinoma moderately differentiated in n=16/30(53.33%) cases followed by SCC well differentiated in n=6/30(20%) cases. There were no cases of squamous cell poorly differentiated carcinoma detected in this study. Similarly n=5/30 (16.67%) were benign lesions n=2/30(6.67%) cases were premalignant and inflammatory lesions and n=1/30(3.33%).

Conclusion: Based on the findings of the current study it can be concluded that oncoprotein p53 overexpression was found in cases with betel quid chewing and smoking and the intensity of expression was directly proportional to the amount of substance consumed or smoked per day. The existence of transforming proteins from viruses, natural radiation, relatively high genetic vulnerability, and other variables may all work in concert to increase the likelihood of malignant transformation in the cells.

Keywords: Oral lesions, Squamous cell carcinoma, p53 oncoprotein, Immunohistochemistry.

Introduction

In the modern world, cancer ranks as the second most prevalent cause of death. Each year, 8 lakh new cases of cancer are recorded in India, of which 48% in men and 20% in women are tobacco-related illnesses that could have been avoided. In India, the incidence of oral cancer is 7.3 for males and 4.3 for women. [1,2] Oral cancers are

thought to be mostly caused by smoking tobacco, drinking alcohol, and chewing betel nuts with or without tobacco. [3] Smoke and smokeless tobacco products both include several carcinogens. These cancer-causing substances react with human DNA to create DNA adducts. These DNA adducts cause mutations and throws off the mechanisms that regulate cellular development during replication. [4] One of the crucial genes involved in cellular proliferation, the p53 gene is also one of the most often altered genes in cancerous tumors. [5] The oncoprotein p53 actively participates in the development of oral cancer, either by initiating or promoting the process. Mutant p53 expression has been observed in various human solid tumors, particularly oral squamous cell carcinomas of squamous origin. [6] The normal, or wild-type, p53 functions as a tumor suppressor gene that is repressed, and its inactivation can result in the transformation of cells into a malignant state. The wild-type p53 protein appears to halt the cell cycle at the GUS boundary, allowing ample time for DNA repair and causing cell cycle arrest in the GI phase. [7] Conversely, the mutant form of p53 forms complexes with the wild-type protein, rendering it inactive and interfering with its regular functions.

This might ultimately result in malignant transformation due to anomalies in the cell cycle repair mechanism. Malignant transformation may result from p53 gene mutations giving the cells a growth edge over healthy ones. [8] In comparison to normal or hyperplastic oral epithelia, more cells are likely to have a high nuclear staining response because the mutant oncoprotein p53 appears to play a role in malignant transformation. It is yet unclear what role the oncoprotein p53 plays in the development of head and neck tumors. To gain insight into the molecular specifics of the process, more research addressing the potential participation of this oncoprotein, such as Direct Nucleotide Sequencing, Polymerase Chain Reaction (PCR), and Single Strand Conformation Polymorphism (SSCP), is necessary. This study examines the expression of p53 in oral cavity lesions and its associations with tobacco use, including eating betel nuts with or without tobacco.

Material and Methods

This cross-sectional study was conducted in the Department of Pathology (hematology unit) of RVM Medical Institute of Medical Sciences and Research Center, Laxmakkapally Village, Mulugu, Siddipet, Telangana State. Institutional Ethical Committee approval was obtained for the study. Written consent was obtained from all the patients or parents/guardians of the cases in the study.

Inclusion criteria

1. History of tobacco intake/smoking habits
2. Oral lesions Leukoplakia, Erythroplakia, Oral submucous fibrosis, and suspected malignancy
3. Adequate histological sample availability.

Exclusion criteria

1. Specimens not sent in formalin.
2. Specimens with inadequate history.

We obtained a comprehensive record of the medical history of individuals who exhibit leukoplakia, erythroplakia, mucosal ulcer, growth, or pain in the upper respiratory and digestive systems. Gather detailed information about the personal history of patients, including their smoking habits, betel nut chewing, and consumption of pan or gutkha, specifying the total duration, frequency per day, and the amount consumed daily. Assess the tissue samples by performing histopathological analysis using hematoxylin and eosin-stained slides. Additionally, conduct P-53 immunostaining and evaluate the tissue samples. From the clinical case records, the demographic profile and pertinent clinical

history, such as age, sex, habitual history, etc., were documented for each patient. The biopsy samples that have been received are fixed in 10% neutral buffered formalin. Surgical grossing was performed following the established procedure after sufficient fixation, which is typically 24 hours, and a thorough gross description was provided. To look for suspicious locations, extensive sampling is carried out. Tissue slices are then extracted, processed manually as needed, and then embedded in paraffin. Three slices measuring 4-6µ in thickness were cut, two of which underwent hematoxylin and eosin staining to determine Anneroth grading, and one of which underwent immunohistochemical staining for p53 using autoimmunostainer (benchmark) to evaluate its expression. On IHC-stained sections, the analysis of p53-positive cells was carried out. Only the nuclei of epithelial cells were stained, and no matter how intensely stained they were, nuclei with a distinct brown hue were considered to be p53 positive. The slide was scanned from beginning to end, excluding any places with no marking at all. The slides were inspected until a total of 1000 nuclei were found, and only the proportion of cells expressing p53 positively was measured in this investigation the number of cells. [9]

Statistical analysis: All the available data was uploaded to an MS Excel spreadsheet and analyzed by SPSS version 19 in Windows format. The continuous variables were expressed as mean and standard deviations and percentages. The categorical variables were expressed as p values. Fischer's Exact test was used to determine the differences between two variables p values of (<0.05) were considered as significant.

Results

In this study based on the inclusion and exclusion criteria, n=30 cases were selected and analyzed. Out of the n=30 cases n=11(36.67%) were females and n=19(43.33%) were males. Out of the cases tobacco chewing habit was found in n=26 cases out of which n=15/26 were males and n=9/26 were females. Smoking habit analysis found out of n=17 smokers in the study n=15/17 were males and n=2/17 were females (Table 1).

Table 1: Distribution of habits in the cases of the study

	Frequency	Percentage
Tobacco Chewing Habit		
No	04	13.33
Yes	26	86.67
Smoking Habit		
Smoker	17	56.67
Non-Smoker	13	43.33

Age-wise distribution of smoking habits revealed maximum cases were between the age group of 31 – 50 years with 64.70% of all the cases included in the study details have been depicted in Table 2. Similarly, for the tobacco chewing habit, the maximum numbers of cases were between the age group 31 – 50 years with 20/26 (76.92%) cases (Table 3).

Table 2: Age-wise distribution of smoking habits

Age in years	Smoking Habits		Total
	Non-Smokers	Smokers	
21 – 30	2	3	5 (16.67%)
31 – 40	3	7	10 (33.33%)
41 – 50	4	4	8 (26.67%)
51 – 60	3	2	5 (16.67%)
> 60	1	1	2 (6.67%)
Total	13	17	30 (100%)

Table 3: Age-wise distribution of Tobacco chewing

Age in years	Tobacco chewers		Total
	Non-Chewers	Chewers	
21 – 30	1	4	5 (16.67%)
31 – 40	2	11	13 (43.33%)
41 – 50	0	9	9 (30.0%)
51 – 60	1	2	3 (10.0%)
> 60	0	0	0 (0.00%)
Total	4	26	30 (100%)

In the current study, we found over-expression of p53 in 25/30 cases of lesions of the oral cavity. The immunostaining reactions for oncoprotein p53 were confined to the cell nuclei. Well-differentiated tumors expressed strong immune staining for p53, and keratin pearls remained negative for staining. Among the n=25 cases of positive lesions, n=12 showed strong staining and n=9 showed moderate staining and n=4 showed weak staining depicted in Table 4.

Table 4: Sex distribution of p53 staining

The intensity of p53 staining	Females	Males	Total (%)
Nil	3	2	5 (16.67)
+	2	2	4 (13.33)
++	3	6	9 (30.00)
+++	3	9	12 (40.00)
Total	11	19	30 (100.0)

In the current study based on the histopathological diagnosis most common lesion was squamous cell carcinoma moderately differentiated in n=16/30(53.33%) cases followed by SCC well differentiated in n=6/30(20%) cases. There were no cases of squamous cell poorly differentiated carcinoma detected in this study. Similarly, n=5/30 (16.67%) were benign lesions n=2/30(6.67%) cases were premalignant and inflammatory lesions and n=1/30(3.33%) was negatively depicted in Figure 1.

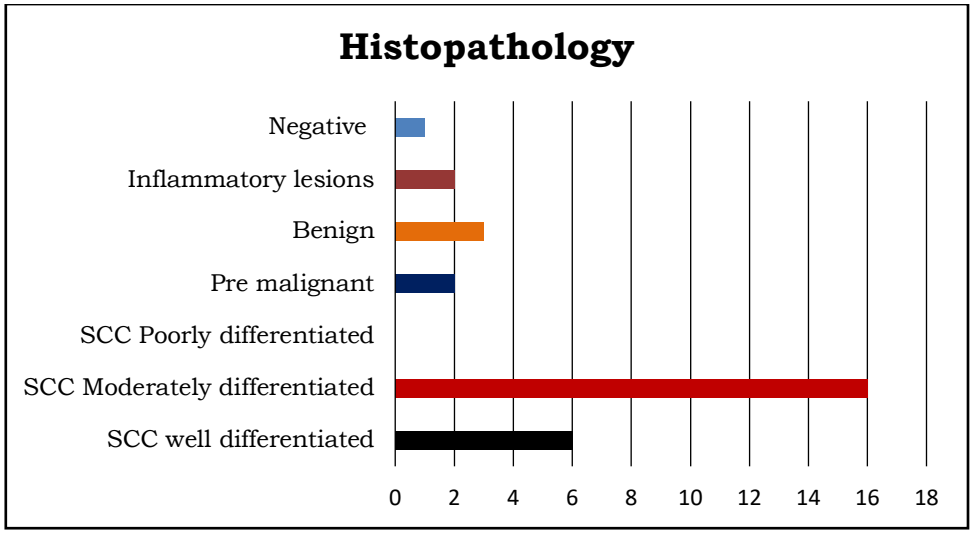

Figure 1: Histopathological diagnosis of lesions in the study

In the current study, we found most of the cases of squamous cell carcinoma were found to be p53 positive, and in non-malignant cases, the n=6/8 were found to be p53 negative depicted in Table 5.

Table 5: p53 marker status in Squamous cell carcinoma and non-malignant lesions

Histopathological diagnosis	p53 Negative	p53 positive	Total
Squamous cell carcinoma	1	21	22
Non-malignant	6	02	08
Total	7	23	30

In the current study, we found most of the cases of squamous cell carcinoma were found to be p53 positive, and in non-malignant cases, the n=6/8 were found to be p53 negative depicted in Table 5.

The analysis of the intensity of staining of p53 in cases of squamous cell carcinoma has been depicted in Table 6. Most of the cases of well-differentiated squamous cell carcinoma and moderately differentiated carcinoma were showing strong positive for p53 staining.

Table 6: p53 staining intensity in squamous cell carcinoma

Squamous cell carcinoma	P53 staining intensity		Total
	Negative/weakly positive	Strongly positive	
SCC well differentiated	1	4	06
SCC Moderately differentiated	2	15	16
SCC poorly differentiated	0	0	00
Total	3	19	22

The histopathological diagnosis of the different lesions and their relationship with p53 staining intensity is represented in Table 7. Out of the lesions, the SCC well differentiated showed the highest intensity. Similarly, 50% of cases of moderately differentiated showed moderate intensity and high-intensity staining. All the benign,

premalignant, and inflammatory lesions were found to be negative or weakly positive for p53 staining given in Table 7.

Table 7: p53 staining intensity among different lesions in the cases of the study

Histopathological diagnosis	P53 staining intensity				Total
	-	+	++	+++	
SCC well differentiated	1	1	1	4	06
SCC Moderately differentiated	1	1	8	6	16
SCC poorly differentiated	-	-	-	-	00
Premalignant	1	1	-	-	02
Benign	2	1	-	-	03
Inflammatory lesions	2	-	-	-	02
Negative	1	-	-	-	01

The oncoprotein's expression did not significantly correlate with the lesion site, according to the current investigation. The participants in the majority of the p53-positive instances had a history of chronic chewing, including chewing and smoking. In the case of chewers, a statistically significant association was found between the staining intensity and the quantity of betel quid consumed daily. The p53 oncoprotein overexpression revealed a modest preference for men, and the majority of them smoked and chewed tobacco.

Discussion

In the current study, we evaluated the p53 expression in oral lesions and it was found that oncoprotein p53 expression did not significantly correlate with the site of the lesion. The cases in a majority of the p53-positive instances had a history of chronic chewing, including chewing and smoking. In the case of tobacco chewers, a statistically significant association was found between the staining intensity and the quantity of betel quid consumed daily. The p53 oncoprotein overexpression revealed a modest preference for men, and the majority of them smoked and chewed tobacco. Oral squamous cell carcinomas have been observed to overexpress the p53 oncoprotein. Additionally, p53 staining intensity has a linear relationship with rising dysplasia levels and the emergence of SCC as shown in other studies. [10] Since p53 oncoprotein was found in more than 70% of the cases examined here, p53 mutations probably contribute to the development of oral malignancies from dysplasia to squamous cell carcinomas. A mutation in one of the alleles with the subsequent loss of the remaining allele throughout time is another possibility. Malignant transformation may result from a mutation in one of the alleles and/or loss of the remaining allele, according to some research. [11] The fact that oral Squamous cell carcinomas exhibit more than 40% of cells that bind to proliferating cell nuclear antigen (PCNA) suggests that malignancy may be facilitated by an expansion of the cell cycle in some way connected to the expression of the p53 oncoprotein. [12] According to a study nonsense mutation, which results from changes in nucleotide sequences that turn triplet codons for specific amino acids into termination codons, and/or deletions, which are not detectable by immunohistochemistry, account for less than 10% of the immunohistochemically detectable p53 mutations. [13] The higher positive p53 staining in oral carcinomas may be partially explained by the loss of function of the wild-type allele and/or the presence of missense mutations.

This study analyzed the antigen retrieved using an immunohistochemistry method. It appears that the mutant proteins that have acquired a dominant transforming activity remain in the nucleus since the protein's staining response is restricted to the nucleus. [14] Multiple genetic alterations are necessary before a normal cell becomes cancerous, according to the theory of step-wise carcinogenesis. [15] Human neoplasia, particularly head and neck, and oral squamous cell carcinomas, are etiologically influenced by substances like alcohol and cigarettes. Additionally, bladder cancer [16] and lung cancer [17] have been linked to tobacco use. Studies have shown that p53 plays an important role in oral cancers in habitual tobacco betel quid chewers the expression was linear with the amount of consumption of quid per day. [18] Studies by Xiong et al., [19] have demonstrated that cells taken from patients with Li-Fraumeni Syndrome (LFS), a syndrome that represents an inherited familial disorder with a comparatively high susceptibility for early onset of various tumors, [20] exhibit loss of p21 protein from CDK complexes. The remaining wild-type p53 allele may also experience mutation in LFS patients, who already possess a copy of the p53 mutant allele, [21] and whose cells do not contain any known DNA viruses. [22] Therefore, the deletion of the p53 gene may affect the cell cycle's regular operation as well as the activities of other cell cycle-associated proteins that work together to facilitate malignant transformation (49). P21 may depend on the p53 pathway to alter cell proliferation and CDK complexes, or that p21 itself mediates the normal tumor suppressor activity of p53. [24] The upper GI tract mucosa may predispose to cancer as a result of the genotoxic effects of alcohol, cigarettes, and dietary variables, which may then predispose the whole epithelium to malignant transformation. [25] The current study found greater percentages of cases were p53 positive, in the patients with a history of tobacco intake/smoking habit. However, this correlation was statistically significant (p-value <0.05). However, these results need further verification with a larger sample size, and cases distributed evenly between both groups.

Conclusion

Based on the findings of the current study it can be concluded that oncoprotein p53 overexpression was found in cases with betel quid chewing and smoking and the intensity of expression was directly proportional to the amount of substance consumed or smoked per day. The existence of transforming proteins from viruses, natural radiation, relatively high genetic vulnerability, and other variables may all work in concert to increase the likelihood of malignant transformation in the cells. Squamous cell carcinomas are promoted and/or progressed by the p53 oncoprotein, and p53 mutations have been found in human squamous cell carcinomas.

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