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# p53 Codon 72 Gene Polymorphism Studies and p53 Expression by IHC in Oral Lesions as Risk Factor for Malignancy

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## Abstract

**Background:** Wild type p53 nuclear phosphoproteins are critical cell cycle regulatory tumor suppressor gene. Genetic mutation of p53 gene is common in several Head - Neck cancers, usually associated with smoking and HPV infection. In India, instead of HPV, tobacco/pan masala chewing are more commonly associated with oral cancer, hence the present study was planned.

**Materials and Methods:** A total of 41 cases of oral lesions comprising 6 cases of leukoplakia and 35 cases of oral SCC, between 30-60 years age, and were tobacco/pan masala chewers were taken. Analysis of p53 codon 72 gene polymorphism was performed by PCR - RFLP for Arg/Arg, Arg/Pro and Pro/Pro. Tissue expression of p53 was done by IHC.

**Results:** Genotype frequencies of 35 carcinoma cases of p53 Arg/Arg, Arg/Pro and Pro/Pro were 23%, 57% and 20% respectively and 6 leukoplakia cases of p53 Arg/Arg, and Arg/Pro genotype were 50% and 50% respectively. By IHC for expression of P53 out of 35 cases of OSCC biopsies 17(48.57%) had weak staining, 14 cases (40%) showed evidence of P53 protein staining and 4 cases (11.42%) showed negative staining. Among 6 cases of Leukoplakia, 3(50%) showed weak staining and 3(50%) showed negative results.

**Conclusion:** The findings of the present study indicate that there is no significant association between P53 codon 72 gene polymorphism and p53 expression by IHC with OSCC and Leukoplakia, associated with tobacco/pan-masala chewing.

**Keywords:** Oral squamous cell carcinoma; Gene polymorphism; p53 codon

## Abbreviations

OSCC - Oral squamous Cell Carcinoma; IHC – Immunohistochemistry; HPV - Human Papilloma Virus; SNP - Single Nucleotide Polymorphism; PCR - Polymerase Chain Reaction; RFLP - Restriction Fragment Length Polymorphism; CIS - Carcinoma-in-situ.

## Introduction

Squamous cell carcinoma of oral cavity and pharynx are the 6th most common cancer in worldwide distribution [1].There is a wide variation in the incidence and mortality rates of oral cancer in different regions around the world. As per latest data 9 per 1,00,000 individuals suffer from oral and lip cancers. 90% of oral cancers are of the squamous cell variety, commonly involving tissue of the mouth and tongue. Betal quid, bidi smoking, alcohol and tobacco are common risk factors involved in oral carcinogenesis. In India cancer registries have confirmed a high incidence of oral cancer and case control and cohort studies have established that the high incidence is due to widespread use of tobacco chewing. Smoking and alcohol consumption it ranks among the top three types of cancer in Indian subcontinents [2-5] Age adjusted rates of Oral cancer in India is 20 per 100,000 population and accounts for over 30% of all cancers in the country [6]. Oral cancers develop through a series of histopathological stages from mild/low grade disease to severe/high grade lesions to Carcinoma in situ (CIS) and finally invasive disease. Prevalence of precancerous condition also has increased substantially because of intense promotion and marketing of few forms of tobacco products so that more chances of further increase in the incidence of oral cancer is envisaged. It has been seen that development of squamous cell carcinoma can be a multifactorial process associated with various risk factors for oral cancer development, only some smokers, alcohol users and betal quid users develop oral cancer. These environmental exogenous carcinogens play a crucial role in the development of oral cancer either by altering the expression of tumor suppressor gene, apoptosis or may result in genomic instability by inducing a defective DNA damage response [5,6]. Early detection of oral premalignant lesion can prevent development of invasive cancers but till now no useful study is available for the same.

In recent years much emphasis has been given on the polymorphism of p53 Codon 72 gene. p53 is a tumor suppressor gene, located on chromosome17p13.1, which plays a role in cell cycle progression, cellular differentiation, DNA repair and Apoptosis that is why it is also known as guardian of the genome. p53 is the most

commonly mutated gene and is altered in over 50% of all cancers, including 25-70% of oral cancers. The aim of this study was to investigate p53 Codon 72 gene polymorphism and expression of p53 by IHC (Immunohistochemistry) in oral lesions as a risk factor for its association with malignancy.

## Materials & Methods

This study was carried out in the Department of Pathology, Era's Lucknow Medical College and Hospital. 50 Patients of all age groups attending out-patient department of Era's Lucknow Medical college and Hospital and Surgical Oncology department of KGMU, Lucknow with any complaints related to oral cavity lesions excluding the dental problems, were examined clinically. A detail history was taken. Blood and biopsy from the representative sites of oral lesions were collected with informed consent. All the biopsy sections were studied for expression of P53 was done by IHC, for P53 expression.

For DNA extraction approximately 3 ml venous blood was collected from each of the aforesaid patients and controls. Blood was mixed slowly with ethylenediamine tetracetic acid (EDTA) for 1 minute and stored at -200°C. Genomic DNA was isolated from whole blood using the standard phenol-chloroform extraction method. The DNA concentration was determined by Spectrophotometer and stored at -200°C. Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis was conducted to identify the p53 polymorphism in codon 72.

### Analysis of polymorphism

Polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis was conducted to identify the p53 polymorphism in codon 72 with the primers 5' ATCTACAGTCCCCCTTGCCG-3' and 5'-GCAACTGACCGTGCAAGTCA-3' [7]. The PCR reaction was performed in 25 µl volumes containing 50 ng of genomic DNA template, 12.5 pmol of each primer, 0.1 mM of each deoxynucleoside triphosphate, 1 PCR buffer (50 mM KCl, 10 mM Tris-HCl and 0.1% Triton X-100), 1.5 mM $MgCl_2$ and 1.5 U of Taq polymerase (Promega Corporation, Madison, WI). PCR conditions were follows: an initial denaturation step at 94°C for 4 min, 35 cycles of 94°C for 40s, 56°C for 30s and 72°C for 30s; and a final extension at 72°C for 10 min.

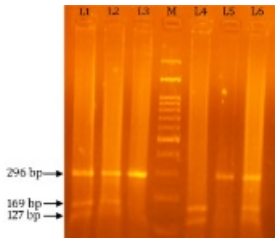


**Figure 1:** PCR-RFLP products belonging to the polymorphism of p53 gene codon 72 obtained by agarose gel electrophoresis. M is 100bp DNA markers; Heterozygous arginine/proline genotype bands of 296,169 and 127 bp (Lane 1,2,6); Homozyggous proline genotype, a single band of 296bp (Lane 3,5); and Homozygous Arginine genotype bands 169, 127bp (Lane 4)

Then, the PCR product (a 296 bp fragment) was digested by BstUI (New England BioLabs, Beverly, MA) overnight at 60°C and then analyzed on 2.5% NuSieve 3:1 agarose gel (FMC BioProducts, Rockland, ME) with ethidium bromide and photographed with Polaroid film. The p53 72Pro allele, which lacked the BstUI restriction site, had only a single 296 bp band, whereas p53 72Arg, which had the BstUI restriction site, produced 169 and 127 bp bands (Figure 1). More than 10% of the samples were retested randomly, and the results were 100% concordant.

### Statistical analysis

All the statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) version 16 software. The genotyping data were compared between cases and controls using Chi-square test. P-values ≤0.05 were considered as significant.

## Observation and Results

All the demographic details and clinico-pathological characteristics of cases included in the study are shown in table 1. Out of 41 cases included in present study, 34 (82.92%) were males and 7(17.07%) females. All cases were categorized into three subgroups like 17 cases (41.46%) of less than 40 years, 19 cases (46.34%) between 41-55 years and 5 cases (12.19%) of more than 60 years. Highest percentage of OSCC patients was identified between 40-60 years. Regarding the primary site of lesion the predominance of buccal mucosa 26 patients (63.41%) was noted, followed by tongue 9 patients (21.95%) and low percentage was observed for palate, that is in 6 patients (14.63%). The clinical staging of tumor was done (usually numbers I to 1V) to know how much tumor had spread. In the present study, stage III disease showed highest frequency (43.90%) as compared to stage II (26.82%), stage I (14.63%) and stage IV (0%). Histopathologically out of 41 cases of oral lesions 35 cases (85.36%) were squamous cell carcinoma and 6 cases(14.63%) of Leukoplakia. We have also done grading of OSCC out of 35 cases of oral squamous cell carcinoma, maximum numbers were well differentiated 29 cases (70.73%) as compared to moderately differentiated 6 cases (14.63%) and no case of poorly differentiated OSCC was found. Out of 35 cases of OSCC biopsies 17(48.57%) had weak IHC staining for p53, 14 cases (40%) showed evidence of p53 protein staining and 4 cases (11.42%) showed negative staining (Figure 2&3). Among 6 cases of Leukoplakia, 3(50%) showed weak staining and 3 (50%) showed negative results (Table-1).

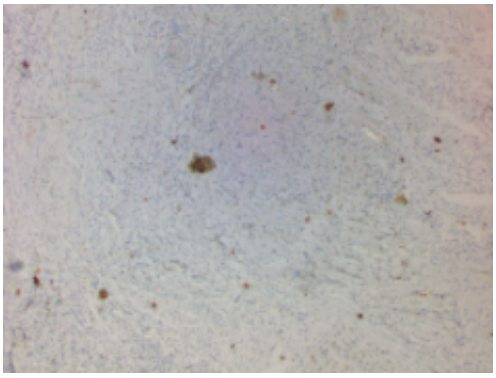

**Figure 2:** Showing weak expression of P53 by IHC (10x)

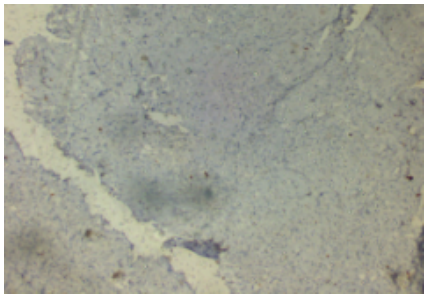

**Figure 3:** Showing Negative expression of P53 by IHC (10X)

| Demographic Characteristics | Cases N=41 (OSCC; n=35 Leukoplakia; n=6) | Frequency |
|---|---|---|
| **Gender** | | |
| **Males** | 34 | 82.92% |
| **Female** | 7 | |
| **Age (Years)** | | |
| **<40** | 17 | 41.46% |
| **41-55** | 19 | 46.34% |
| **>60** | 5 | 12.19% |
| **Site of lesion** | | |
| **White patches buccal mucosa** | 26 | 63.41% |
| **Tongue** | 9 | 21.95% |
| **Ulcer hard palate** | 6 | 14.63% |
| **Histopathology** | | |
| **Squamous cell carcinoma** | 35 | 85.36% |
| **Leukoplakia** | 6 | 14.63% |
| **Grading** | | |
| **Well differentiated** | 29 | 70.73% |
| **Moderately differentiated** | 6 | 14.63% |
| **Not applied (Leukoplakia** | - | |
| **Staging** | | |
| **Stage-I** | 6 | 14.63% |
| **Stage-II** | 11 | 26.82% |
| **Stage-III** | 18 | 43.90% |
| **Stage-IV** | - | |
| **Not Applied (Leukoplakia)** | - | |

**Table 1:** Demographical details of individuals included in the study

## Distribution of p53 genotypes/alleles in oral squamous cell carcinoma, carcinoma, leukoplakia patients and controls

In the present study it was observed that frequency of Arg/Arg genotypes was lower in OSCC patients as compared to controls (P<0.720), whereas frequency of Arg/pro and Pro/Pro was elevated in OSCC patients as compared to controls (P<0.630). In Leukoplakia patients, frequency of Arg/Arg, Arg/Pro & Pro/Pro genotype was equal to controls (P<0.548). It was observed in present study that codon 72 polymorphism, was not associated significantly neither with OSCC nor with Leukoplakia, associated with tobacco/pan masala consumption (Table 2&3).

| Allele/ Genotype | OSCC (n= 35 ) | Frequency (%) | Controls (n=15) | Frequency (%) | P-Value |
|---|---|---|---|---|---|
| **Arg/Arg** | 8 | 23% | 5 | 33% | 0.720 |
| **Arg/Pro** | 20 | 57% | 7 | 47% | 0.8097 |
| **Pro/Pro** | 7 | 20% | 3 | 20% | |

**Table 2:** Distribution of the P53 codon 72 alleles and genotypes in OSCC samples and controls.

| Allele/ Genotype | Leukoplakia (n= 6) | Frequency (%) | Controls (n=6) | Frequency (%) | P-Value |
|---|---|---|---|---|---|
| **Arg/Arg** | 3 | 50% | 3 | 50% | 0.548 |
| **Arg/Pro** | 3 | 50% | 2 | 34% | 0.548 |
| **Pro/Pro** | 0 | 0% | 1 | 16% | |

**Table 3:** Distribution of the P53 codon 72 alleles and genotypes in Leukoplakia samples and controls.

## Discussion

Development of cancer is a multifactorial and multi-step process which occurs with an effect of series of progressive genetic alteration. Various genetic studies at molecular level states that a group of proto-oncogenes and tumor suppressor genes alterations were play an effective role in cancer development. Among group of tumor suppressor genes, p53 is the most important one which play an important role in various cancers including Oral Squamous Cell Carcinoma. Hence detection of p53 mutations is an important factor for early diagnosis and treatment of OSCC. Uptill now, various studies have provided evidence that p53 Polymorphism at codon 72 may be associated with certain cancers like Breast Carcinoma [8], Lung Cancer [9], Hepatocellular Carcinoma [10] and Oesophageal Carcinoma. Specifically both Arg and Pro alleles have been found to be associated with high risk of development of cancer. The present study has been planned out to evaluate the gene polymorphism of p53 codon 72 and its correlation with p53 expression on IHC, which form an important tool for future early diagnostic modalities in treatment of Oral Cancers.

Occurrence of a single nucleotide polymorphism(SNP) at codon 72 of p53 leads to the presence of either Arg or Pro alleles, which in turn could result in three different genotypes; Arg/Arg, Arg/Pro, Pro/Pro. The structure of the p53 protein is affected by substitution of the Arg codon with Pro or vice versa, although the mechanism by which this might affect function of p53 remains controversial.

The effect of p53 codon 72 polymorphism has been debated in various carcinomas. For example Storey et al. have proved that overexpression of homozygous Arg 72 p53 protein can increase the susceptibility of cervical cancer which is HPV associated upto seven fold [11]. By contrast, Lin and Colleagues have proved that Pro 72 p53 allele is associated with increased risk of lung squamous cell carcinoma and adenocarcinoma [12]. In other study Twu and Colleagues, it has been found that the heterozygous Arg/Pro genotype is associated with an increased risk of hypopharyngeal squamous cell carcinoma [13]. These findings have also been supported by some other studies [14-17], while they have also opposed by some other investigators [18-19].

There is limited results on the role of p53 codon 72 polymorphism on OSCC. In a study carried out in Taiwan, Bau and Colleagues reported that the Arg/Arg genotype seems to increase the risk of OSCC by 2.7 folds [20]. By contrast, in present study we found no significant association between p53 codon 72 polymorphism genotypes and OSCC. Our observation is similar to the results obtained by Shen and colleagues on head and neck squamous cell carcinoma [21] as well as Katiyar et al. on HPV associated oral cancer in India [22]. These variations in results might be possible due to role of various factors such as geographic distribution and racial differences on various predisposing factors involving tobacco consumption, betel quid, bidi smoking, alcohol use, lifestyle and risk of HPV virus. The major limitation in the present study was small sample size. Thus additional studies with larger sample size might be required in near future to further evaluate the role of p53 codon 72 gene polymorphism as a risk factor for the development of oral squamous cell carcinoma.

## Conclusion

The present study was undertaken with a view to assess the association of p53 codon 72 gene polymorphism and p53 expression by IHC as a risk factor for the development of OSCC in patients of oral lesions. Our results showed a lack of association between p53codon 72 gene polymorphism and OSCC susceptibility. The negative results might be due to small sample size, therefore additional studies with larger sample size are required in near future to investigate the role of various environment and thenic factors in order to determine the role of p53codon 72 gene polymorphism in OSCC.

## Conflicts of Interest

The author declares that they have no Conflicts of interest.

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