Section: Dental surgery

Original Research Article

ASSESSMENT OF P53 AT CODON 72 POLYMORPHISM IN ORAL LESIONS: A CASE CONTROL STUDY FROM COASTAL ANDHRA PRADESH.

Received : 07/11/2023
Received in revised form : 20/12/2023
Accepted : 05/01/2024

Keywords:
p53, polymorphism, PCR- RFLP, oral lesions and OSCC.

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DOI: 10.47009/jamp.2024.6.1.427

Source of Support: Nil,

Conflict of Interest: None declared
Int J Acad Med Pharm
2024; 6 (1); 2142-2144

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Abstract

The p53 protein modulates the genomic stability of cells in a variety of ways. In human cancers, disruption of p53 activity is frequently observed. The p53 codon 72 polymorphism is believed to play a key role in the susceptibility to oral cancer. The study was aimed to investigate the association of p53 codon 72 polymorphism with oral leukoplakia in coastal Andhra Pradesh population. The case-control study conducted in 100 healthy controls and 115 patients who were attended to dental OPD and were clinicopathologically confirmed oral carcinomas in Machilipatnam region of Andhra Pradesh, India. The study was conducted in period between October 2023 and January 2024. Using the PCR-RFLP method, genotyping was done between 100 controls and 115 patients with oral lesions. The distributions of p53 codon 72 genotypes between patients and controls differed significantly, according to our findings. The p53 Arg/Arg, Arg/Pro, and Pro/Pro genotype frequencies were 91%, 16%, and 3% in the controls and 65%, 31%, and 19% in the patients with oral lesions, respectively. Proline/Proline (P<0.002) and arginine/proline (P<0.001) genotypes were higher in patients than in controls, while arginine/arginine was higher in controls than in patients (P<0.0001). Compared to proline, the frequency of the arginine allele was higher in instances. According to the current study's findings, the p53 codon 72 polymorphism may increase the risk of oral squamous cell carcinoma in the population of Machilipatnam, Andhra Pradesh, India.

INTRODUCTION

The p53 protein, acting predominantly as a transcription factor, is a renowned tumor suppressor protein with dual functions: halting the cell cycle and triggering apoptosis following genotoxic stress. Mutations in key regions of the p53 gene leading to loss of tumor suppressor function are significant in promoting cellular transformation.[1] The necessity of the proline rich domain of p53 for its apoptotic and growth suppressive capabilities has been well documented. With this domain, a common polymorphism at position 72 results either an arginine or proline residue, with each variant allele displaying distinct biochemical and biological activities.[2] Various theories have been put forth to elucidate the impact of the Arg allele on cancer progression, with its weaker affinity for certain transcription- activating process being a key factor. Furthermore, research has demonstrated that the Arg allele displays increased vulnerability to degradation by the human papillomavirus (HPV) E6 protein compared to the Pro allele in living organism. This allele may also strengthen mutant p53's interaction withp73, consequently inhibiting p73- induced cell death in squamous cell carcinoma, regardless of HPV-related pathways.[3,4] A statistically significant association between 72 Pro/Pro frequency and latitude has been found in many populations.[5,6] These observations suggest that Pro/Pro and Arg/Arg alleles can produce different proteins and that the allele encoding Pro/pro may be selected in an environment exposed to high UV irradiance.[5]

MATERIALS AND METHODS

A combined total of 115 individuals diagnosed with oral squamous cell carcinoma and 100 age-matched controls were included in the study, with sample collection taking place at Government General Hospital, Machilipatnam, Andhra Pradesh, from October 2023 to January 2024. 4 ml blood samples were obtained from the pathology lab following the diagnosis of oral cancer, and were collected for each

ISSN (O): 2687-5365; ISSN (P): 2753-6556

patient. Additionally, blood samples were collected from healthy individuals through venipuncture to serve as controls.

Genotyping of p53 codon 72 polymorphism

Genomic DNA was extracted from whole blood according to the protocol used by Addala et al. [7]. PCR amplification was performed using the primer sequences primer forward – 5'-TTCACCCATCTACATCC-3' and reverse primer - 5'- CTCAGGCAACTGACCGT -3', and 25µL of PCR reaction mix containing 0.2mM of dNTP mix, 1.5mM MgCl2 and 2U taq DNA polymerase and taq buffer (1X). Thermalcycler conditions used in this assay was denaturation at 94°C for 30sec, annealing at 55°C for 30 sec, and extension at 72°C for 30sec. and performed 2% agarose gel to check the PCR amplification. RFLP was done using the BstUI (Bsh1236I) restriction enzyme (Fermatas, USA). After RFLP, a PCR product of 309 base pair (bp) was indicating Pro/Pro, two bands at 175 and 134bp indicating Arg/ Arg, and three bands of 309, 175, and 134 bp indicating arginine/proline1 and 2 bands. Statistical analysis was performed using Medical (v.10.3.1) statistical software.

RESULTS

An analysis of the association between tumor suppressor gene p53 polymorphisms and oral cancer was conducted by examining the polymorphic status of the p53 codon 72 through allele-specific PCR in a cohort of 115 oral cancer patients, revealing genotype frequencies of 65% for the arginine/arginine allele, 31% for the heterozygous arginine/proline allele, and 19% for the proline/proline allele. The genotype and frequency percentages of cases and controls can be found in Table 1.

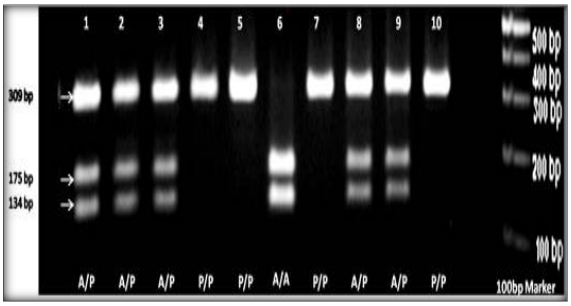

Figure 1: P53 Codon 72 polymorphism RFLP Gel picture

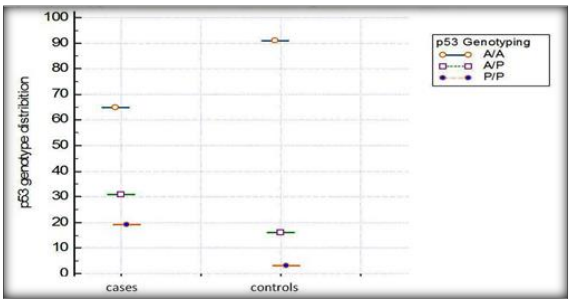

Figure 2: Genotype Distribution between Patients and Controls

Table 1: Genotype Distribution between Controls and Patients

p53 Genotyping	Cases (n = 115)	Controls (n = 110)	Odds Ratio	95% CI	P-Value
A/A	65 (56.5%)	91 (82.7%)	0.36	0.18-0.7	0.0041
A/P	31 (26.9%)	16 (14.5%)	0.30	0.07 to 1.19	0.0876
P/P	19 (16.5%)	3 (2.7%)	0.1128	0.03 to 0.39	0.0007

Significance: P<0.0001

A graphical representation of the genotype distribution between patients and controls is shown in Graph 1.

DISCUSSION

Cancer is a complex process that arises from a sequence of genetic alterations during development.[8] Molecular genetic studies have shown that mutations in a group of tumor suppressors and proto oncogenes are causative agents in the progression of cancer. It has been accepted that p53 plays important role in cell cycle control, DNA repair and apoptosis, thus affecting tumor formation, growth and response to DNA damage. The p53 codon 72 polymorphism causes proline (Pro) to replace arginine (Arg) in the p53 protein sequence, thus it can function at the cellular level, affecting cell apoptosis potential and cell arrest in the G1 phase of the cell cycle. In this study of 115 patients with oral cancer, we found that p53 Arg/Pro heterozygotes (31%) were at risk of malignancy together with Arg/Arg homozygotes (65%). The different functions of arginine and proline alleles may affect cancer risk by affecting DNA repair capacity, apoptosis and chromosomal susceptibility to mutagens.

Detection of p53 mutations is important for clinical and early diagnosis. Recently, many studies have provided evidence that p53 codon 72 polymorphism may be associated with some other cancers like lung cancer,[9] hepatocellular carcinoma,[10] and carcinoma breast.[11] In particular, both Arg and Pro alleles have been revealed that the associated with a higher risk of malignancy. This study evaluated the association between the risk of oral cancer and the genotype of codon 72 of p53 gene. Controls have more Arg/Arg genotypes than cases, and according to the previous data, the Pro allele is more common in the general population. In our study, the frequency of the Arg allele was higher than the Pro allele in both the cancer and control groups.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Previous studies have investigated the association of this polymorphism with oral cancer and reported and increased risk of Arg/Arg oral cancer compared to Taiwanese.[12] and Germans.[13] Arginine variants have been reported to be more effective than proline variants in inducing apoptosis due to differences in protein localization in mitochondria.[14] Homozygous arginine at codon 72 does not appear to play a significant role in the development of oral cancer. This specific genetic variation is not considered a functional biomarker for the early diagnosis of oral cancer.[15] The study also found a 2.50 fold and 3.51- fold increase risk of OSCC in individuals with Arg/pro and Pro/Pro, indicating an association between Arg/Pro and Pro/Pro codon 72 p53 polymorphism. Several studies have shown that proline substitutions are more effective than TP53-dependent DNA repair genes in different cellular based assays.

The upregulation of Pro72 in tumors led to a reversal in the expression of certain proteins, resulting in enhanced apoptosis induction. The presence of the Arg allele in Arg/Pro heterozygous tumors seems to confer protection against apoptosis. It is clear that there is a correlation between the genotype of codon 72 and the initiation of apoptosis. The Arg72 allele enhances p53's ability to localize to mitochondria and trigger cell death, whereas the Pro72 allele demonstrates diminished apoptotic potential and cell survival.

The prevalence of oral cancer is defined by the growth of malignancies in the gums, tongue, hard palate, and lips. Previous studies have demonstrated a significant association between p53 codon 72 polymorphisms and specific head and neck cancers, including laryngeal cancer,[17] and nasopharyngeal carcinoma.[16] Approximately 2,000 chemicals are found in cigarettes, many of which are directly linked to the development of cancer. Early cancer recurrence and the development of secondary tumors have been connected to the high frequency of p53 mutations, which are frequently linked to alcohol and cigarette use.[18] Our study revealed that individuals with prolonged exposure to the pro allele exhibited a greater likelihood of chewing tobacco over smoking cigarettes.

CONCLUSION

In conclusion, it is thought that codon 72 polymorphism have different functions and their expression also changes the cancer risk. The results of this study suggests that p53 polymorphism at codon 72 may be a genetic risk factor for oral squamous cell carcinoma and p53 Arg72 protein may be associated with cancer risk in Machilipatnam population.

Acknowledgement

I thank all the faculty members who supported in this study.

Conflicts of interest: No conflicts of interest.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556