**P53 Codon 72 Gene Polymorphism and Risk of Oral Squamous Cell Carcinoma in South Indian Population: A Case-Control Study**

Lakshmi Addala¹, Kalyan Kumar Ch², Mohan Reddy N³, Anjaneyulu V⁴ and Sadanani MD¹

¹Institute of Genetics, Osmania University, Hyderabad-500034, A.P, India
²Indo American Cancer Institute and Research Centre, Banjara Hills, Hyderabad-500034, A.P, India
³MNH Cancer Hospital, Red Hills, Hyderabad-A.P, India

**Abstract**

**Background:** The p53 protein has pleiotropic functions in the modulation of genomic stability of cells. Disruption of p53 activity is commonly found in human cancers. The p53 codon 72 polymorphism is likely to play an important role in the susceptibility to OSCC. We aimed to investigate the association of p53 codon 72 polymorphism with oral squamous cell carcinoma in south Indian population.

**Methods:** We genotyped 150 OSCC patients and 150 controls, using PCR-RFLP method.

**Results:** Our results showed a significant difference in the distributions of p53 codon 72 genotypes among cases and controls. Genotype frequencies of p53 Arg/Arg, Arg/Pro and Pro/Pro were 50.8, 33.9 and 14.4% in the oral squamous cell carcinoma patients and 48.7, 45.1 and 6.2% in the controls, respectively. Arginine/arginine genotype was elevated in controls compared to patients (P<0.0001), where as Proline/proline (P<0.005) and arginine/proline (P<0.002) genotypes were elevated in patients compared to controls. Arginine allele frequency showed high in cases than Proline.

**Conclusion:** The results of the present study detected that p53 codon 72 polymorphism may contribute to oral squamous cell carcinoma susceptibility in south Indian population.

**Keywords:** Oral squamous cell carcinoma; Arg72Pro; PCR-RFLP; Polymorphism; TP53

**Introduction**

Oral squamous cell carcinoma (OSCC) is one of the most common malignant neoplasms worldwide. Oral cancer forms a large part of the cancer load in the part of India [1]. Smoking, alcohol, drinking, viral infection and genetic factors are the major risk factors involved in the etiology of oral squamous cell carcinoma. Some molecular studies have provided some clues to the molecular mechanisms underlying such genetic susceptibility, and it is likely that they are genetically determined. DNA repair capacity may contribute to interindividual variation in susceptibility to head and neck carcinogenesis. The tumour suppressor gene p53 is crucial for host defence against genomic mutations which might cause many types of tumours. The p53 protein has pleotropic functions in the modulation of genomic stability of cells. Disruption of p53 activity is commonly found in human cancers. The production of p53 is increased in response to cellular insults or DNA damage, and p53 then induces cell cycle arrest at the G1/S junction. If the damage is irreparable, p53 can initiate cell death by apoptosis [2]. Mutant proteins are defective in DNA binding in a sequence-specific manner, and thus in the upregulation of downstream genes [3]. So far 13 polymorphisms have been described in this gene [4]. The most commonly studied one is a single nucleotide polymorphism (SNP) at codon 72 in exon four of the p53 gene, which results in the substitution of arginine (Arg) by proline (Pro) in the transactivating domain. These two polymorphic variants (Pro72 and Arg72) alter the structure and function of the p53 protein [5]. The potential consequence of this amino acid exchange is differences in the susceptibility to malignant transformation, induction of apoptosis, and transcriptional activity [6]. The arginine (Arg72) allele increases the ability of p53 to locate to mitochondria and induce cellular death, whereas proline allele (Pro72) exhibits a lower apoptotic potential and an increased cellular arrest in G1 of the cell cycle [7].

Several epidemiological surveys reported association and non-association of this codon 72 (Arg/Pro) polymorphism with different types of cancers [8], it suggests that Pro72 Pro genotype may be the potential risk factor favoring the development of lung carcinoma in Kerala population and Arg72Pro genotype is independently associated with a poorer prognosis of lung cancer. De Chaudhuri, et al. [9], in a study reported that individuals carrying the arginine homozygous genotype at codon 72 and no duplication homozygous genotype at intron three are at risk for the development of arsenic induced keratosis. Rajkumar et al. [10], study reported that SNP’s can help predict breast cancer risk in south Indian women. Sailaja et al. [11], suggests that proline genotype might confer increased risk to develop CML in Hyderabad population and is also associated with poor cytogenetic response. In Mumbai population equidistribution of the genotypes were detected in both normal controls and oral cancer patients. Thus Tandle et al. [12], in a study reported that Association of p53 genotypes with oral cancer was not detected. In contrast, to above investigations investigators in some studies demonstrated the non-association between the different p53 polymorphisms and individual cancer development, other studies revealed the higher risks in the individuals with p53*Pro72 homozygote. The aim of the present study is to identify whether TP53 codon 72 polymorphism can be considered...
as risk factor for developing oral squamous cell carcinoma in south Indian population and its impact on disease progression.

Materials and Methods

Study population

Oral squamous cell carcinoma patients were assessed on the basis of clinical and pathological examinations. This study is a Hospital-based case-control study conducted on South Indian population. All incidents of OSCC cases are newly diagnosed during the study period, Ethical committee approved the study. The procedures followed according to the ethical standards of responsible committee of the Institutes/Hospitals, to participate in a face-to-face interview using a structured questionnaire.

Selection criteria

Senior pathologists confirmed all diagnoses. We interviewed and collected the demographic factors data from the patient. We collected the information on age, smoking, chewing, usual alcohol intake, and previous cancer diagnoses. Participant’s family history of cancer and the clinical information for these cases are obtained from medical records, tumor size, stage, and chemotherapy drugs. Patients were recruited based on inclusion and exclusion criteria, which were determined before the beginning of the study.

Inclusion and exclusion criteria

All new cases of clinically confirmed oral squamous cell carcinoma would be taken for study. Patients of confirmed oral carcinoma who give their consent were included. Patients who refuse to give consent and cases of oral cancer other than squamous cell carcinoma are excluded.

Sample collection

A total of 150 OSCC patients and 150 age-matched controls are enrolled in the study. Sampling was done from one major hospital, MNJ cancer Institute in Hyderabad, Andhra Pradesh between the periods June 2008 to March 2010.

Collection of blood samples

3 ml of blood samples were collected from pathology lab after diagnosis. All the samples diagnosed mainly as oral squamous cell carcinoma. Blood sample were collected from healthy individuals (voluntarily) by venipuncture. These samples are used as controls.

Genotyping of the P53 codon 72 polymorphism

Genomic DNA was extracted from whole blood according to standard procedures used by our group [13]. PCR amplification was carried out using following primers, 5’ TTCACCCCATCTACAGTCC 3’ and 5’ TCTAGGGCAACTGACCGT 3’ synthesized at Bioreverse Biotechnologies (Hyderabad, India). Three steps PCR amplification was performed as described earlier, briefly a 25 µl reaction was set up containing 0.2 mM of each dNTP, 1X buffer, 1.5 mM MgCl$_2$ and 2U of Taq DNA polymerase (Bioreverse, India). 30 cycles were performed with denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and elongation at 72°C for 30 seconds. The PCR amplification was checked on a 3% agarose gel. 10 µl PCR reaction mix was subsequently digested with BstUI (Bsh1236I) restriction enzyme (Fermentas, USA). PCR product of 309-bp indicating Proline/Proline after digestion gave two bands of 175bp and 134bp showing Arginine/Arginine and three bands of 309, 175 and 134 bp indicating Arginine/Proline (Figure 1 and 2). Statistical analysis was performed using Medical (Version 10.3.1) statistical software (Belgium).

Results

Biological characteristics

The distribution patient’s biological characteristics and selected risk factors are shown in Table 1. Age range for OSCC patients was 9-87 years in males and 27-75 in years in females and in controls 21-80 years in males and 22-87 years in females. However, many of the ages mentioned in case sheets or given by patients were arbitrary, exact age of 150 OSCC patients (Males 81 and Females 69) and 150 controls (Males 91 and Females 59) was available, hence analysis was carried out with those, mean age at which OSCC identified as 9-87/49.30 ± 15.55 in males and 27-75/84.20 ± 11.26 in females years. To understand the role of gene mutations/ polymorphisms in onset of the disease, the patients were divided into 4 categories, <25 years (1.33%), 26 to 45 years (32.00%), 46 to 65 years (54.00%), and above 66 years (20.12%). Highest percentage of OSCC patients was identified between 46-65 years. Regarding the primary tumor site, there was a neat predominance on the BM adding up 56 patients (37.33%), followed by tongue adding up 33 patients (22.0%), then mandible, oral cavity and RMT adding up 12%, 10% and 7%. In the present study, a neat predominance on the BM adding up 56 patients (37.33%), followed by tongue adding up 33 patients (22.0%), then mandible, oral cavity and RMT adding up 12%, 10% and 7%. In the present study, Stage III showed the highest frequency (40%) when compared to Stage II (31.33%), and other types of tumor grade like Stage I (6.67%) showed very low frequency when compared to other staging groups. The percentage of patients with family history was 5% in oral squamous cell carcinoma patients In the present study majority of patients 40.7% had received radiation therapy.

with adjuvant chemotherapy, 23.3% underwent surgery, followed by surgery with adjuvant chemotherapy 14.0%, 13.3% and 0.7% of the patients received radiation and chemotherapy alone. Surgical excision and/or adequate radiation therapy remain the most effective means of treating the patients with OSCC.

Distribution of p53 genotypes/alleles in OSCC patients and controls

In the present study, OSCC patients showed 58.66% GG, 26.66% GC and 14.66% CC genotypes when compared to controls that had 82.66% GG, 12.66% GC and 4, 6% CC genotypes. It was observed that codon 72 polymorphism was significantly associated with OSCC. The frequency of arginine/arginine genotype was elevated in OSCC in controls compared to patients (P<0.0001), where as frequency of arginine/arginine (OR. 3.51, 95% CI 1.45-8.49, P<0.005) and arginine/proline (OR. 2.50, 95% CI 1.37-4.57, P<0.002) genotypes were elevated in patients compared to controls (Table 2).

P53 genotypes and clinocopathological characteristics of oral squamous cell carcinoma

We further stratified the associations between the p53 genotypes and age, site, stage smoking and alcohol drinking to understand the influence of p53 codon 72 polymorphism on the OSCC risk. The frequency of Arginine allele was found to be high in median age group (46-65) patients when compared to age group among 26-45 patients. In this study, we confirmed that Buccal Mucosa patients were found to be with high frequency of Arginine when compared with proline. According to the 1997 TNM staging system, 60 patients presented with stage IV, and 47 with stage III disease. For patients with stage IV disease, frequencies of the Arg/Arg, Arg/ Pro and Pro/Pro genotypes were 30.6%, 25.0% and 45.45%, respectively. For patients with stage III disease, the frequencies of the Arg/Arg, Arg/Pro and Pro/Pro genotypes were 39.7%, 50.0% and 22.7%, respectively. We observed that stage 3 and stage 4 patients with high frequency of p53 codon 72 polymorphism. Our study between the p53 genotypes and habitual risk factors like chewing and smoking confessed that the frequency of Arg/ Arg (OR: 1.01, 95% CI: 0.31-3.68) Arg/Pro, (OR: 1.40, 95% CI: 0.27-7.13) & Pro/Pro (OR: 2.75, 95% CI: 0.31-24.1) genotype were high in chewers than smokers. Arginine allele frequency was detected to be high (OR: 1.04, 95% CI: 0.34-3.21) in alcohol+chewing patients when compared to alcohol+smoking patients. However, the interaction between p53 genotypes and age, site of diagnosis, tumor stage, and habitual risk factors was not statistically significant (Table 3).

Discussion

Cancer is a multi-step mechanism occurring as an effect of series of progressive genetic alterations. Molecular genetic studies revealed that a group of tumor suppressor gene and proto-oncogene alterations were effective in cancer formation. p53 is the most important tumor suppressor gene which is effective in different cancers including oral squamous cell carcinoma. Detection of p53 mutations is an important factor in prognosis and early diagnosis.

Recently, several studies have provided evidence that p53 polymorphism at codon 72 may be associated with certain tumors, such as esophageal, lung cancer [14], hepatocellular carcinoma [15] and breast cancer [16]. In particular, both Arg and Pro alleles have been shown to be associated with a high risk of malignancy. This study evaluates the association between the risk of developing oral squamous

<table>
<thead>
<tr>
<th>P53 Genotyping</th>
<th>Cases (n = 150)</th>
<th>Controls (n = 150)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>216 (72%)</td>
<td>287 (89%)</td>
<td>0.31</td>
<td>0.20-0.49</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pro</td>
<td>64 (28%)</td>
<td>33 (11%)</td>
<td>3.1</td>
<td>2.02-4.88</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>88 (58.66%)</td>
<td>124 (82.66%)</td>
<td>0.29</td>
<td>0.17-0.50</td>
<td>0.0001</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>40 (26.66%)</td>
<td>19 (12.66%)</td>
<td>2.50</td>
<td>1.37-4.57</td>
<td>0.002</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>22 (14.66%)</td>
<td>7 (4.66%)</td>
<td>3.51</td>
<td>1.45-8.49</td>
<td>0.005</td>
</tr>
</tbody>
</table>

p = < 0.05 (Significant) OR: Odds Ratio, 95% Confidential Intervals, Chi Square.

Table 2: Distribution of p53 genotypes/alleles in OSCC patients and controls.

Table 3: Association between TP53 codon 72 status and clinicopathological characteristics.

<table>
<thead>
<tr>
<th>P53 Genotyping</th>
<th>Males (n = 81)</th>
<th>Females (n = 69)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>46</td>
<td>42</td>
<td>OR: 0.84, 95% CI: 0.43-1.62, P = 0.61</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>22</td>
<td>18</td>
<td>OR: 1.05, 95% CI: 0.51-2.18, P = 0.88</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>13</td>
<td>9</td>
<td>OR: 1.27, 95% CI: 0.50-3.19, P = 0.60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P53 Genotyping</th>
<th>Age Between 26-45 (n = 48)</th>
<th>Age Between 46-65 (n = 81) Above 66</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>29</td>
<td>46</td>
<td>OR: 1.16, 95% CI: 0.56-2.40, P = 0.68</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>12</td>
<td>21</td>
<td>OR: 0.95, 95% CI: 0.41-2.16, P = 0.90</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>7</td>
<td>14</td>
<td>OR: 0.81, 95% CI: 0.30-2.19, P = 0.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P53 Genotyping</th>
<th>BM (n = 56)</th>
<th>Tongue (n = 33)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>33</td>
<td>19</td>
<td>OR: 1.05, 95% CI: 0.44-2.52, P = 0.90</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>15</td>
<td>7</td>
<td>OR: 1.35, 95% CI: 0.48-3.77, P = 0.55</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>8</td>
<td>7</td>
<td>OR: 1.61, 95% CI: 0.20-2.19, P = 0.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P53 Genotyping</th>
<th>Stage IV (n = 47)</th>
<th>Stage III (n = 60)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>35</td>
<td>27</td>
<td>OR: 1.03, 95% CI: 0.47-2.24, P = 0.92</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>20</td>
<td>10</td>
<td>OR: 1.85, 95% CI: 0.76-4.46, P = 0.17</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>5</td>
<td>10</td>
<td>OR: 3.70, 95% CI: 0.89-15.3, P = 0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P53 Genotyping</th>
<th>Chewing (n = 46)</th>
<th>Smoking (n = 12)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>27</td>
<td>7</td>
<td>OR: 1.01, 95% CI: 0.31-3.68, P = 0.98</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>9</td>
<td>4</td>
<td>OR: 1.40, 95% CI: 0.27-7.13, P = 0.68</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>10</td>
<td>1</td>
<td>OR: 2.75, 95% CI: 0.31-24.1, P = 0.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P53 Genotyping</th>
<th>Alcohol/Chewing (n = 35)</th>
<th>Alcohol/Smoking (n = 19)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>17</td>
<td>9</td>
<td>OR: 1.04, 95% CI: 0.34-3.21, P = 0.93</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>13</td>
<td>6</td>
<td>OR: 1.28, 95% CI: 0.39-4.19, P = 0.68</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>5</td>
<td>4</td>
<td>OR: 0.62, 95% CI: 0.14-2.67, P = 0.52</td>
</tr>
</tbody>
</table>

Oral cancers occur in patients with tumors arising from the gingival, tongue, hard palate, and lip. However, previous studies of specific types of head and neck cancers usually found positive association with p53 codon 72 polymorphism, such as nasopharyngeal carcinoma.
[26] and laryngeal tumors [27]. In the present study, Buccal Mucosa patients showed high frequency of p53 codon 72 polymorphism. Tobacco contains 2000 chemicals many of which are have been directly related to causing cancer. p53 mutations commonly arise as a result of alcohol and tobacco exposure, and their presence is associated with the early recurrence and development of second primary tumors [28]. We revealed that delayed stage patients with pro allele showed high frequency with the effect of chewing when compared to smoking. The relationship between age, gender & habitual risk factors are not statistically significant with p53 codon 72 polymorphism in south Indian population.

Conclusion

In conclusion, codon 72 polymorphism were thought to function distinctly from one another and their expression levels also modulated cancer risk. The findings of the present study indicate that p53 codon 72 polymorphism may be a genetic predisposing factor for oral squamous cell carcinoma and p53 Arg72 protein may be correlated with possible increased risk of this kind of cancers in south Indian population.

References


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