C Deletion in Exon 4 Codon 63 of p53 Gene in Turkish Patients with Oral Squamous Cell Carcinoma

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OBJECTIVE
Oral squamous cell carcinoma (OSCC) is the most frequently seen oral malignancy and accounts for up to 80-90% of all malignant neoplasms that occur in the oral cavity. The p53 tumor suppressor gene plays a crucial role in the regulation of the cell cycle. Mutations of the p53 gene have an important role in OSCC carcinogenesis. In this study, we aimed to evaluate the C-deletion mutation in exon 4 codon 63 of p53 gene in Turkish patients with OSCC.

METHODS
A total of 60 subjects were enrolled in this study, 30 patients with a pathologic diagnosis of OSCC and 30 cases of age and sex-matched healthy controls. Genotyping was performed for all individuals using polymerase chain reaction (PCR) analysis.

RESULTS
The findings showed that the distribution of p53 exon 4 codon 63 C-deletion was significantly different between patient group and control group (p=0.000). It was detected that all patients had C-deletion mutation in exon 4 codon 63 of p53.

CONCLUSION
Our results suggest that C-deletion in exon 4 codon 63 deletion of the p53 gene may play a role in the pathogenesis of human OSCC in a Turkish cohort.

Keywords: Deletion; oral squamous cell carcinoma; p53; PCR.

Introduction
Oral cancer broadly consists of tumors developing in the lips, hard palate, upper and lower alveolar ridges, anterior part of the tongue, sublingual region, buccal mucosa, retromolartrigone and base of the mouth.[1] Squamous cell carcinoma is the major histological type (seen in ~95% of the cases). Thus, the term ‘oral cancer’ is mostly used interchangeably with oral squamous cell carcinoma (OSCC).[2] The three main carcinogenic stimuli, chemical, physical (radiation), and infectious (oncogenic viruses) agents, may cause mutations in
the oral cavity which may modify the structure of the
genes and chromosomes.[3] Mutations may lead to
deregulation and constitutive activity, overproduction
of a normal gene product, or changes in the biochemi-
cal function of the gene product.

p53 gene covers 16-20 kb of DNA on the short
arm of the chromosome 17 and consists of 11 exons
and acts as a tumor suppressor gene. p53 functions
as a ‘guardian of the genome’ to sustain the balance
of cell death and proliferation by modulating the cell
cycle, DNA repair, apoptosis, cellular metabolism and
senescence.[4,5] p53 genetic inactivation is mostly as-
cribed to its conformation mutations and allelic dele-
tion. Mutant p53 not only acts as a tumor suppressor
but can also exhibit tumor-promoting effects. Mutated
gene encodes for a mutant protein with a higher cellular
stability.[6] These mutations are generally found in
the exons 5-8 of the p53 gene and are related with poor
prognosis in some human tumors, such as oral, lung,
and breast, prostate and colorectal tumors. Mutation in
the p53 gene is the most common genetic aberration
encountered in oral premalignant lesions and squa-
mous cell carcinoma in situ.[6] Therefore, in this study,
we aimed to determine the C-deletion in exon 4 codon
63 of p53 gene in a Turkish population.

Materials and Methods

Study Population
This case-control study included 30 OSCC patients (18
males and 12 females, mean age: 58.63±12.13 years)
and 30 unrelated healthy controls (19 males and 11
females, mean age: 62.77±12.06 years). All OSCC pa-
tients were histopathologically confirmed and were
recruited from the Department of Medical Oncol-
ogy, Faculty of Medicine, Gaziosmanpasa University
(Tokat, Turkey). The healthy control group was similar
concerning age and sex distribution; and they did not
have any evidence of OSCC or any other oral diseases.
Informed written consent was obtained from all sub-
jects. This study protocol was approved by the Local
Ethics Committees (2017-14/154) in accordance with
the ethical standard for human experimentation estab-
lished by the Declaration of Helsinki.

Genotyping
The DNA of the participants was isolated from periph-
eral blood mononuclear cells using a DNA extraction
kit, according to the manufacturer’s instructions (Sig-
ma-Aldrich, Taukirchen, Germany). The C-deletion
in exon 4 codon 63 of p53 gene was genotyped in all
the subjects by the polymerase chain reaction (PCR)
analysis, as described method previously.[7] PCR re-
action was carried out in a 25 μl reaction volume con-
taining 1 μg/μl of genomic DNA, 2.5 μl of 10X Taq
polymerase buffer with 1.5 mM MgCl2, 200 μM of each
dNTPs,15μg of each primer and 1 unit of Taq DNA
polymerase μl. On the basis of sequence, the primers
were constructed for C-deletion on codon 63 of exon 4
of p53 gene as F- 5’-GGTCCAGATGAAAGTCAGAGAA
and R-5’-CGTGCAAGTCACAGACTTTGCGC. A neg-
ative control, without template DNA was included in
each round of reactions. PCR thermal was performed
in 35 cycles. Each cycle consisted of 94°C denaturation
for 45 s, 57°C annealing for 45 s, and 72°C extension
for 1 min. The thermal cycles were started with an ini-
tial denaturation of 96°C for 5 min and a final 72°C
extension for 10 min for polishing the ends of PCR
products. The thermal cycles were started with an ini-
tial denaturation of 96°C for 5 min and a final 72°C
extension for 10 min for polishing the ends (making smooth)
of PCR products.

Construction of Primer Against C Deletion on Exon
4 of p53 Gene
On the basis of sequence, the primers were constructed
for C deletion on codon 63 of exon 4 of p53 gene as
5’-GGTCCAGATGAAAGTCAGAGAA (upstream) and
5’-CGTGCAAGTCACAGACTTTGCGC (downstream).
The annealing temperature of constructed primers was
estimated as 58°C and the PCR amplification reaction
was done as described above.

Agarose Gel Electrophoresis
Resulting PCR products were resolved (15 μl PCR
product mixed with 2 μl gel loading dye) on 1.5%
agarose gel using submarine gel electrophoresis for one
hour in 1X TBE buffer (Tris HCl, boric acid, EDTA; 
pH 8.0). Subsequently, gels were stained with ethidium
bromide (10 mg/l) and photographed on a UV transil-
luminator using a gel documentation system.

Statistical Analysis
All statistical analyses were performed with the Statis-
tical Package for the Social Science for Windows (ver-
sion 18.0; SPSS Inc., Chicago, IL, USA). Continuous
data were given as means±SD and minimum/maxi-
mum. The χ² test was used to measure significance of
differences in the allele frequency and genotype distribu-
tion between the two study groups. Odds ratio (OR)
and 95% confidence intervals (CI) were calculated. A
p-value≤0.05 was considered statistically significant.
Results

A total of 60 subjects were included in the present study. Age, gender, and age at the time of diagnosis were analyzed. Demographic characteristics of the study participants are shown in Table 1.

The C-deletion mutation in exon 4 codon 63 of p53 gene among the OSCC patients and controls are shown in Table 2. There was a significant difference between OSCC patients and controls. All patients appeared C-deletion exon 4 codon 63 of p53 gene (p=0.000).

Discussion

OSCC is one of the most common oral cancers. Tobacco and alcohol consumption is the most important risk factors for OSCC, but a genetic predisposition has also been implied given that the most of the population exposed to these risk factors do not have oral cancer and that sporadic cases of oral tumors are seen in young adults and nonusers of tobacco and alcohol.

Carcinogenesis is a complicated and multi-factorial process in which genetic events within signal transduction pathways executing normal cellular physiology are changed.[8] Cancer is the result of an amassing of alterations in the excitatory and inhibitory cellular pathways, which may take place at any level of a certain pathway. It is believed that somatic mutations ranging between three to six are required for transforming a normal cell into its malignant one.[8] p53 is an important tumor suppressor gene. This gene found on chromosome 17p 13.1 encodes a 53-kDa, 393 amino acid nuclear phosphoprotein. Wild-type p53 protein functions as a DNA-binding transcription factor and results in the control of the cell cycle by G1/S phase detainment following sublethal DNA damage. Hence, providing extra time for repair prior replication or the induction of apoptosis if the damage is too severe.[9] It is also believed that of over half of human tumors that display mutations in the p53 gene have dysfunctional p53 signaling. The prevalence of p53 mutations also depend on the tumor type. The majority of p53 mutations found in human cancer map to the DNA-binding surface of the p53 protein, comprising two large loops, L2 (residues 163–195) and L3 (residues 236–251) and a loop-sheet-helix motif.[10]

Comprehensive genomic studies have indicated that p53 mutations occur commonly in head and neck squamous cell cancers. Missense, stop-gain, splice site, frameshift deletions, and inframe deletions are among the various types of p53 mutations that are implicated in the early stages during the carcinogenetic process of the corresponding epithelia. Missense and truncating mutations are related with malignant cell proliferation, enhanced invasion, and resistance to chemotherapeutic regimens. All of these histological and biochemical factors play a role in poor prognosis (decreased drug response rates and short survival span), particularly in missense mutations carriers. Lazarus et al. reported that incidence of p53 mutations have been found in approximately 63% of OSCC.[11] Hsieh and Wang et al.reported that mutation in p53 is detected in 48% of tumor samples. [12] Zanaruddin et al. found that p53 mutations were present in 27.7% of the OSCC specimens.[13] A study group analyzed a large number of OSCC tissues and found that p53 mutations are associated with lower survival rates in these patients.[14]

In this study, we investigated the association between C-deletion exon 4 codon 63 of p53 and OSCC in Turkish patients. To our knowledge, this research is the first to study evaluating this deletion in our OSCC patients. We found that all patients had C deletion mutation in exon 4 codon 63 of p53 gene. The patients carrying p53 C deletion had 17.957-fold increased risk for

Table 1 The demographical characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Patient group (n=30)</th>
<th>Control group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.63±12.13</td>
<td>62.77±12.06</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12 (40.0)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>Male</td>
<td>18 (60.0)</td>
<td>19 (63.3)</td>
</tr>
<tr>
<td>Diagnosis age</td>
<td>56.93±9.35</td>
<td></td>
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</tbody>
</table>

Table 2 C-deletion mutation in exon 4 codon 63 of p53 gene in groups

<table>
<thead>
<tr>
<th>C-deletion exon 4 codon 63</th>
<th>Patient group n (%)</th>
<th>Control group n (%)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>30 (100)</td>
<td>16 (53.3)</td>
<td>17.957</td>
<td>0.000</td>
</tr>
<tr>
<td>Negative</td>
<td>0 (0)</td>
<td>14 (46.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
OSCC (p=0.000). High mutation rate may be due to ethnic differences.

There were several limitations to this study. Expression of p53 in biopsy tissue samples was not studied. Secondly, study population represented relatively small sample size. Also, the absence of examination of other mutations regarding p53 is another limitation.

Conclusion

p53 mutations are heterogeneous and act through a complex and intricate network of multiple proteins. Clinically, p53 mutations are crucially linked with poor survival and tumor resistance to radiotherapy and chemotherapy in OSCC patients, which implies that p53 mutations can be used as a marker that can bear prognostic and predictive value concerning clinical response. Also, the development of efficient and long-lasting therapeutic modalities for OSCC patients with tumors having p53 mutations is needed. In this study, all patients had C-deletion mutation in exon 4 codon 63 of p53. Therefore, we think our results are important because they reflect Turkish patient profile. This data may help providing novel treatment options.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethics Committee Approval: This study protocol was approved by the Local Ethics Committees in accordance with the ethical standard for human experimentation established by the Declaration of Helsinki.

Financial Support: This study was supported by Ahi Evran University BAP (SYO.A4.16.002) program.


References