Pathological study of p53 and ki67 in relation to clinic pathological parameters of laryngeal cancer

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Short running title of no more than 40 characters:
p53, laryngeal cancer.

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Abstract

Background: This study aimed to determine the significance of p53, evaluated by immunohistochemistry, and its possible correlation with clinicopathological parameters of laryngeal cancer.

Methods: FFPE (Formalin fixed, paraffin-embedded blocks) from 50 patients with laryngeal cancer were included in this study. We considered the uninvolved vocal cord that have no any pathological changes of thirty cases out of the fifty cases was evaluated as a non-tumoral sample were included as a comparative group. Labeled Streptavidin-Biotin Complex (LSAB) method was employed for immunohistochemical detection of p53.

Results: Our study showed significant p53 positivity among malignant cases (35)cases were positive for p53. P53 showed no positive immunoexpression in normal tissue sections with significant difference from that of malignant cases (p<0.05) . p53 showed no significant difference in relation to age ,sex and stage of the tumor. Furthermore p53 showed significant difference in relation to grade , site ,size(p<0.05).

Conclusion: Based upon the findings of this study, it can be concluded that p53 play an important role in the pathogenesis of laryngeal cancer and supports the evidence of its role in evolution and cell survival of this aggressive tumor . This study recommended
that the blocking of p53 may be a target for blocking the evolution and hence improving the efficacy of anti-cancer therapy against this type of cancer.

**Background:**

The squamous cell carcinoma represents about 90% of malignant tumors of the larynx, being correlated with smoking and alcoholism. Laryngeal squamous cell carcinoma (SCC), the most common malignant neoplasm of the upper airway in adults, accounts for about 1.5% of all cancers. These tumors can be classified anatomically, depending on their position in the larynx: supraglottic, glottic and subglottic cancers. The glottic localization has the best prognosis because of its poor lymphatic drainage, slow tumoral progression and late metastasis \(^{1,2}\). Stage, anatomical location, and histological differentiation of a tumor are gross predictors of clinical outcome, but for cancer of the larynx these prognostic factors have a limited predictive value for identifying a high risk of relapse or death. Therefore, it is important to define new prognostic indicators which may help to recognize the biological behavior of the tumor \(^3\). The exact molecular mechanisms of malignant transformation of the laryngeal mucosa are not clear. It has been suggested that the process of tumorigenesis is based on the accumulation of various stages of genetic damage, which impairs the mechanisms of cell cycle regulation \(^3\). Disruption of two major pathways controlling the cell cycle, tumor-suppressor gene p53 and pRb pathway are important \(^4\). The p53 gene product, which is able to block the cell cycle in G1 after encountering genotoxic stress, plays a central role in the G1 phase of the cell cycle \(^3\). Disruption of the pathways regulated by p53 leads to increased cell proliferation and additional genetic damage. Mutations in p53, resulting in expression of a non-functional protein, appear to be the most common abnormality in human neoplasms and are present as an early event in laryngeal carcinoma \(^4\). Although a relation was shown between p53 expression and shorter survival of the patients, conflicting results have been reported regarding its clinicopathological implications in laryngeal cancers \(^5,6,7\). On the other hand, expression of p53 has been associated with progression from dysplastic lesions to invasive carcinoma \(^8,9\).

**Materials and Methods:**

We analyzed tissue samples from 38 men and 12 women with laryngeal SCC (mean age 60 years, age range 40-80 years) at Al Sader teaching hospital. The uninvolved vocal cord was evaluated as a non-tumoral comparative group sample in 30 cases out of 50 malignant cases. All patients were from the same geographic region (middle Euphrates). We selected cases whose disease had been diagnosed at our pathology department and for whom complete medical records were available. None of them had been treated previously. The TNM classifications were based on the 1992 recommendations of the American Joint Committee on Cancer. For conventional histopathologic examination, 10-µm paraffin sections stained with hematoxylin and eosin were examined. Tumors were graded according to morphologic differentiation: grade 1 is well differentiated, grade 2 is moderately differentiated, and grade 3 is poorly differentiated or undifferentiated \(^9\). All specimens were fixed in 10% formalin and routine histologic paraffin sections were made and stained with hematoxylin and eosin. Sections were cut to 3-4-µm thickness and mounted on poly-L-lysine-coated slides. The sections were deparaffinized in xylene and rehydrated in alcohol. The sections were incubated overnight with p53 antigen (pre-diluted, Dako) as primary antibodies. After that, the slides were rinsed gently with phosphate-buffered saline and an Envision Dual
link system-HRP (ready to use, Dako) was used as the secondary antibody. Incubation with 3,3-diaminobenzidine tetrahydrochloride was performed for 10 min as a substrate chromogen solution to produce a brown color. Finally, the sections were counterstained with hematoxylin. Appropriate positive and negative control sections were processed in parallel. Finally, Sections were evaluated under a light microscope and staining intensity of the tumor cells was classified semiquantitatively. P53 staining was considered negative if the staining was below 10% and positive if it was above 10%.\(^{(10)}\)

Statistical analysis: the chi-squared test was used to compare categorical variables. Analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 15; SSPS Inc., Chicago, IL, USA), with \(P < 0.05\) as the cut-off value for significance.

Results: Our study showed significant p53 positivity among malignant cases (70%). P53 showed no positive immunoexpression in normal tissue sections that has been tested in parallel with that of malignant samples, with significant difference from that of malignant cases (\(p=0.000\)). P53 showed no significant difference in relation to age, sex and stage of the tumor (\(P>0.05\)). Furthermore, p53 showed significant difference in relation to grade, size, (\(P=0.003\), \(0.003\) and \(0.02\) respectively. P53 was positive in (82.8%, 55.6% and 83.3%) of (grade I, II and III) respectively. Regarding tumor site it was positive in (80.5%, 25% 20%) of Glottic, Supraglottic and Epiglottic respectively. Furthermore, p53 showed positivity in (77.5% and 40%) of tumor size <4cm and \(\geq 4\) cm respectively. Table(1).

Table 1. p53 overexpression in relation to clinico pathological parameters:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>P53 in relation to clinicopathological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(P53^{+ve})</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>0(-)</td>
</tr>
<tr>
<td>Malignant cases</td>
<td>35(70%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
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<tr>
<td>&gt;50 years</td>
<td>25(71.4%)</td>
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<tr>
<td>(\leq 50) years</td>
<td>10(66.6%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25(65.8%)</td>
</tr>
<tr>
<td>Female</td>
<td>10(83.4%)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
</tr>
<tr>
<td>&lt;4cm</td>
<td>31(77.5%)</td>
</tr>
<tr>
<td>(\geq 4) cm</td>
<td>4(40%)</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
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<tr>
<td>Glottic</td>
<td>33(80.5%)</td>
</tr>
<tr>
<td>Supraglottic</td>
<td>1(25%)</td>
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<tr>
<td>Epiglottic</td>
<td>1(20%)</td>
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Grade
I  29(82.8%)  6(17.2%)  0.003*
II  5(55.6%)  4(44.4%)
III  1(16.7%)  5(83.3%)

Tumor stage
Stage III  25(71.4%)  10(28.6%)  0.74
Stage IV  10(66.7%)  5(33.3%)

*: "significant p value"

Discussion:
It has been suggested that laryngeal tumorigenesis is based on the accumulation of multiple genetic lesions in the sequence of dysplasia-carcinoma. The p53 protein plays an important role in apoptosis and cell growth regulation. This pathway is frequently inactivated by gene mutations that account for increased cell proliferation and makes cells susceptible to additional genetic damage (11).

In previous studies, p53 expression has been found in laryngeal squamous cell carcinoma at frequencies ranging from 40% to 90% (12,13,14). The expression of p53 in the series of patients studied in our study (70%) was within the range of values reported in the literature. In the literature, data on the relationship between p53 and clinicopathological parameters, obtained in different tumor types and from different series, are somehow controversial. p53 showed significant positivity among malignant cases in comparison there was no positive immunoexpression in normal tissue sections.

Fig(1).:(a) Showing positive diffuse p53 expression in squamous cell carcinoma-tumoral-cells.(Immunohistochemical staining (X10).
(b) Showing p53 expression in squamous cell-carcinoma-tumoral-cells.(X40).
(c) Showing negative p53 expression in squamous cell carcinoma tumoral cells.(Immunohistochemical staining .(X40).

a
b
c
this observation was expected to us hence p53 overexpression expression looks to be a landmark in the malignant laryngeal tissue, and does not play any role in the normal and benign laryngeal tissue sections that has been tested in parallel with that of malignant samples, this finding is agreed by Cabanillas R, et al., 2007\(^{15}\).

Our study, showed a statistically significant difference between p53 expression and tumor size, site and grade in patients with laryngeal squamous cell carcinoma. The relation between p53 expression and histological grade, it was positive in (82.8%, 55.6% and 83.3%) of (grade I, II and III) respectively suggests the role of p53 in the progression of laryngeal carcinomas, this finding is agreed with that of Şirin Baspinari et al., 2006\(^{16}\). Furthermore, there was no statistically significant difference between expression of p53 protein in relation to age, sex of the patient (P>0.05), this finding is agreed with that of Mustafa Kazkayasi et al., 2001\(^{17}\). Regarding the tumor stage there was no statistically significant difference between p53 expression in relation to clinical stage, the same finding found in the study of Şirin Baspinari et al., 2006\(^{16}\), in our study this is could be due to the small sample size of IV cases (10 cases) only.

**In conclusion**, we suggest that analysis of p53 expression in tumoral tissues may help predict the clinical course in patients with laryngeal SCC and may, therefore, aid in the selection of patients who should be treated more aggressively.

**References:**


