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The Diagnostic Value of the P53 Tumor Marker as a Prognostic Factor in Patients with Squamous Cell Carcinoma of the Larynx

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ABSTRACT

The objective of this study was to determine expression of the P53 marker in cases of patients with squamous cell carcinoma of the larynx and also to study the possible relationship between the level of the marker and clinical prognostic and histopathologic factors of the disease. It was a prospective analytic epidemiologic study conducted on 42 patients diagnosed with squamous cell carcinoma of the larynx and subjected to laryngectomy. The paraffin blocks of the participants were examined and slides covered with anti-P53 antibodies were subjected to standard immunohistochemistry in order to find the antigen. To assess the level of the P53 marker, grades with scores less than 10% were considered to be negative. In addition, grades with scores between 10% and 60%, 61% and 79%, and ≥80% were also well-differentiated, moderately differentiated, and poorly differentiated, respectively. A total of 35 (83.3%) participants were P53-positive: 28.6% were supraglottic, 48.6% were glottic, and 22.8% were transglottic. In general, there was a significant difference between the level of the P53 marker and the extent and grade of tissue differentiation of the tumor, involvement of blood vessels, invasion of the peripheral nerves, invasion of extrinsic soft tissues of the larynx, distant metastasis. However, no significant difference was observed between the level of the P53 marker and the position of the tumor or involvement of lymph nodes. Surgical margins also showed no signs of positivity of the marker. The P53 marker is a valuable prognostic marker capable of revealing useful information on the clinical growth and position of carcinoma of the larynx.

Key words: P53, Squamous Cell Carcinoma, Larynx, Immunohistochemistry.

INTRODUCTION

Squamous cell carcinoma (SCC) of the larynx is the most common malignant neoplasm of the upper respiratory tract in adults. It accounts for about 1.5% of all malignancies and about 85-95% of all malignant neoplasms of the larynx[1, 2]. The etiologic factors that probably contribute to the onset of squamous cell carcinoma of the larynx include smoking, drinking, laryngopharyngeal reflux (or laryngeal-esophageal reflux), toxins (asbestosis, mustard gas, …), diet (reduction in consumption of

vegetables and increase in consumption of meat and fat), and the HPV virus[3,4]. Although the histopathologic characteristics of the tumor provide information that are important to the prognosis of the disease, no reliable indicator of the disease is currently available that can definitely determine the invasive or recurrent nature of the disease[3, 4, 5].

Factors influencing prognosis of the disease, which were enumerated previously, include characteristics of the tumor, involvement of blood vessels, invasion of the peripheral nerves, extrinsic soft tissues of the larynx, local muscles, and involvement of surgical margin. It is worth mentioning that tumoral characteristics include microscopic findings as well as macroscopic findings, which include tumor size and tumor location (supraglottic, glottis and subglottic). Other factors include involvement of lymph nodes and distant metastasis of other organs[3, 4, 5, 6, 7]. Since tumors with similar clinical and histopathological characteristics may show different clinical behaviors, additional factors must be taken into account in the prognosis of the disease[3, 4].

The P53 marker is a tumor antigen that hosts mutations. It is also one of the most common alterations observed in human cancers[8]. It is suggested that tumor growth is caused by various phases of genetic damage that can lead to disorderliness in the mechanisms of cell cycle regulation[9]. The product of the P53 gene is able to block the cell cycle in phase G1 following to the development of genotoxic stress. It, therefore, plays the chief role in phase G1 [5]. Disorder in the main cell cycle control pathway, which is caused by mutations of tumor antigens, is of great importance as well. Facture of the pathway by P53 brings about increased cell proliferation and genetic damages. Mutation of P53 leads to the expression of a non-functional protein, which is known as the most prevalent abnormality among human neoplasms. It is also one of the earliest manifestations of larynx cancer[10]. Several studies have reported on the significance of the relationship of P53 expression with short-term survival of patients and advancement of lesions from the dysplastic to the carcinoma invasive phase[11,12,13,14]. Several other researchers have made unsuccessful attempts to prove the existence of a substantial relationship between P53 expression and prognosis in patients with squamous cell carcinoma of the larynx. Hence, supplementary studies are required.

The objective of the present study is to determine the diagnostic value of the P53 tumor marker, as a prognostic factor, in tumoral cases of squamous cell carcinoma of the inside of the larynx or its surgical margins. It is also focused on the analysis of a possible relationship between expression of the marker and prognostic factors of the disease.

Methods

It is a prospective analytic epidemiologic study conducted on patients visiting Department of Otolaryngology of Ahvaz University of medical sciences from 2010 to 2012, who have been diagnosed with squamous cell carcinoma of the larynx and have been subjected to laryngectomy. The group of patients under study included 42 candidates, who were selected for laryngectomy after initial checks, examinations and paraclinic measures. Moreover, patients whose histopathologic information did not reveal squamous cell carcinoma of the larynx were excluded from the study. For the purpose of the study, paraffin blocks obtained from patients were trimmed into 5-6μm slices and then the paraffin contents were neutralized using hydrogen peroxide and were washed with water. Next, slides were washed with PBS (phosphate buffered saline) and were incubated with the first anti-P53 antibody (mouse monoclonal Ab). The slides were afterwards washed again and incubated with the second antibody (biotinylated Ab). Following to this phase, the slides were washed with PBS and incubated in DAB. In the next stage, the slides were colored with hematoxylin and dehydrated. In order to estimate the expression of the marker, sample slides were examined using optical microscope (light microscope) at 400x magnification. After examining a thousand cells from each sample, samples were graded on the number of their colored cells. Grade scores less than 10% were considered to be negative, and grade scores between 10% and 60%, 61% and 79%, and ≥ 80% were considered to be well-differentiated, moderately differentiated and poorly differentiated respectively. Following to the data collection phase, the research data were put

into SPSS (version 18) software by a statistics specialist and were subjected to the Pearson's chi square test. Next, the relationship between prognostic parameters and the level of the P53 marker was determined for using the possible significance of the relationship in planning treatment and follow-up programs.

RESULTS

Among 42 patients suffering from larynx cancer, 37 were male and 5 female. Analysis of the differentiation of the tumor indicated that 27 (64.3%) cases were well-differentiated, 10 (23.8%) were moderately differentiated, and 5 (11.9%) were poorly differentiated. A total of 83.3% of the cases (35 patients) were P53-positive: 28.6% supraglottic, 48.6% glottis, and 22.8% transglottic.

The average levels of marker expression in the poorly differentiated group, the moderately differentiated group, and the well-differentiated group were approximately 78%, 68%, and 40%, respectively. The difference was shown to be significant by the Pearson's chi square test (P =0.001).

The average levels of marker expression in the vascular invasive cases and non-invasive cases were about 74% and 47%, respectively. The difference was shown to be significant by the Pearson's chi square test (P =0.001).

In the case of invasion of peripheral nerves, the average level of marker expression was about 77%. However, in the case of no invasion of the peripheral nerves it was about 43%. The difference was shown to be significant by the Pearson's chi square test (P =0.001).

In patients with tumors larger than 4 cm the average level of marker expression was approximately 75%. However, in patients with tumors smaller than 4 cm the average level of marker expression was about 46%. The difference was shown to be significance by the Pearson's chi square test (P =0.001).

In the follow-up and systemic examinations, 3 patients were diagnosed with distant metastasis. All of the three patients demonstrated marker expression levels of higher than 80%. The difference was also shown to be significant by the Pearson's chi square test (P =0.001).

In the next phases it was found out that there was no significant statistical difference between the level of the P53 tumor marker, location of the tumor, and involvement of lymph nodes. The surgical margins of no sample also showed signs of positivity of the P53 marker.

DISCUSSION

Morphological studies and findings are not capable of predicting the invasive behavior and position of tumors. Advanced methods of studying the growth of tumors not only are used in prediction of biologic behaviors of tumoral cells, but also help choose better treatment and follow-up procedures. This advantage especially finds importance in the case of patients that are classified into the highly risky group based on clinical observations and the severity and progression of malignancy of their disease. Hence, these patients need more attention and follow-up examinations.

The P53 tumor marker plays an important role in apoptosis and regulation of cell growth. According to the available theories, carcinogenesis in the larynx is caused by accumulative effects of several mutated genes and occurs in the course of advancement from dysplasia to carcinoma. Due to the importance of the P53 marker in regulation of cell growth, mutation in the marker makes the cells vulnerable to various genetic damages. This study was focused on the P53-based analysis of the growth and proliferation of tumoral cells in patients with carcinoma of the larynx. In sum, about 83.3% were shown to be P53-positive. This finding complies with results obtained other researchers. In their studies, Sherman E and Osman I. *et al*., introduced P53 as an important prognostic factor[15].

In the present study, it was concluded that there is a strong relationship between the level of P53 and differentiation grade of the tumor. In the study by Ziadr the level of P53 was also higher inpoorly-differentiated tumors[16]. However, the

results of the study by Kazkayasi, klatka and Rodriguez revealed no relationship between increase in the P53 marker and tumor differentiation[17, 18, 19].

In the present study, the level of the P53 marker was considerably increased in patients experiencing vascular invasions as well as invasions of the peripheral nerves and frontal soft tissues of the larynx. The increase reflects a strong relationship between the marker and the aforementioned disorders. It is therefore concluded that the P53 factor can be used as an important prognostic factor.

Contrary, Klatka, Rodriguez *et al*, and Xia *et al*, based on separate studies concluded that the P53 factor cannot be considered an important prognostic factor[17, 19, 20]. In the study by Pinar *et al*. it was found out that the P53 tumor marker is not related to common prognostic parameters for squamous cell carcinoma of the larynx[21].

In the present study, however, a direct relationship was found between the level of the P53 marker and tumor size. Similarly, Golusipiki *et al* observed a direct relationship between the level of P53 and tumor size[22]. Moreover, Sedat *et al* and Rashad *et al* concluded that there is significant relationship between expression of P53 and onset of squamous cell carcinoma of the larynx[23, 24]. In the present study, the level of the P53 factor increased considerably in patients suffering from lymphatic invasion, but this difference was not statistically significant. Liu *et al* and Rodriguez *et al* suggested that there is no significant relationship between the level of P53 tumor marker and involvement of lymph nodes[17, 25]. Conversely, Golusipiki reported that the relationship of the P53 marker level with tumor size and involvement of lymph node is direct[22]. The present study also indicated that there is no significant relationship between increased levels of the P53 factor and tumor location. The results of the present research are in accordance with the results obtained by Kazkayasi[18]. However, Ashrafi *et al* reported that the relationship of expression of P53 with tumor location and involvement of lymph nodes is significant. No relationship was observed between expression of P53 and histological findings of studies of non-tumoral cases[26]. In the present study, no significant increase was observed in the expression of the P53 marker in patients with distant metastasis (3 cases). Dolcetti *et al* indicated that dysplastic areas and areas with in-situ lesions were P53-positive. They also asserted that positivity of the marker is actually the first phase of the onset of squamous carcinoma of the larynx[27].

On the other hand, it was reported that tumors with engaged surgical margin showed higher levels of P53[28].

In the present study, none of the non-tumoral tissues showed signs of expression of the P53 marker and in fact were proofs of the research hypothesis. Moreover, surgical margins of none of the samples showed signs of positivity of the marker.

CONCLUSION

Study of the effect of oncogenes on cell growth and apoptosis yields useful information on the biological behavior of tumors. Furthermore, results of the present study indicate that P53 is a valuable tumor marker, which is capable of revealing useful information on the clinical growth and position of carcinoma of the larynx. Hence, analysis of the biologic invasion of the tumor, especially in relation to other clinical-morphological parameters and consequently determining the invasiveness or malignancy of the tumor is a necessity.

Based on the findings of the present study, it is recommended to use the marker in predicting the clinical progress of carcinoma of the larynx and choosing patients who need more invasive treatments. The marker is also useful when histopathology report (biopsy report) of a patient shows pre-malignant lesions in the larynx. In such occasions, the marker is used for making more precise follow-up decisions.

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