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P63 expression in sputum specimen of the patient with non small cell carcinoma

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A B S T R A C T

Lung cancer is the main cause of mortalities resulted from cancer. After prostate cancer in men and breast cancer in women this malignancy is the most prevalent type of cancer among humans. Lung cancer is mostly diagnosed in its advanced stages and therefore its prognosis is often hard. The aforementioned studies stress the importance of expression of P63 in the tissue samples of different types of lung cancer. The aim of this research is to study the expression of P63 in the sputum samples of non-small cell lung cancer patients. In a cross-sectional descriptive analytical study which was carried out in the pathology department of Tabriz University of Medical Sciences on patients with lung cancer, the expression of P63 in the sputum samples of patients with non-small cell lung cancer was investigated. In this research, the expression of P63 in the sputum samples of 50 patients with non-small cell lung cancer and 50 healthy individuals was examined and compared. The mean age of non-small cell lung cancer patients and members of the control group was 63.20 ± 15.01 and 62.04 ± 12.50 years, respectively (P=0.676). The mean expression of P63 in the non-small cell lung cancer patients and control group participants was 32.40 ± 19.98 and 2.48 ± 4.09 percent, respectively. The mean expression of P63 in non-small cell lung cancer patients was significantly higher than the control group (P<0.001). Mean of P63 expression in patients with lung SCC was 39.80 ± 19.39 percent, in patients with Adenocarcinoma was 25 ± 17.79 percent, in patients with Carcinoid atipic was 18 ± 12.54 percent and in patient with undifferentiated carcinoma was 60 percent. Mean expression of P63 was significantly higher in patients with lung SCC (P=0.010). Mean expression of P63 in patients with well differentiated tumor was significantly higher than patients with moderately differentiated tumor (P=0.018).

Introduction

Lung cancer is the main cause of mortalities resulted from cancer. After prostate cancer in men and breast cancer in women this malignancy is the most prevalent type of cancer among humans. Lung cancer is mostly diagnosed in its advanced stages and therefore its prognosis is often hard. Hence, it is necessary to diagnose this cancer in its early stages when treatment is still feasible. Moreover, most patients also suffer from cardiopulmonary diseases because of smoking. As a result, surgical and non-surgical procedures are harder to implement for these patients (1).

Non-small cell lung cancer accounts for approximately 75% of lung cancer and includes Adenocarcinoma (35-40%), squamous cell carcinoma (SCC) (25-30%), and large cell carcinoma (10-15%)(2).

The difference between the aforementioned types lies in the histological findings and clinical characteristics because they have similar treatment and prognosis (2).

Adenocarcinoma of the lung is the most common type of lung cancer. It is normally developed in the peripheral areas of the lung. The origins of its tumor are the mucous glands in bronchioles and it is usually manifested in the form of scar-carcinoma. This type of lung cancer is mostly seen in non-smokers and its bronchoalveolar type may be multifocal (3).
The small-cell carcinoma type of lung cancer is different from the aforementioned type in its clinical and biological characteristics, prognosis and curability. This type of lung cancer is invasive, advances quickly and leads to metastasis. On the other hand, it is highly sensitive to chemotherapy and radiation and is further accompanied by remote paraneoplastic syndromes. Surgery is rarely used for the treatment of this type of lung cancer (4).

Recently, advancements in molecular techniques have indicated that activation of some oncogenes and inactivation of tumor suppressors play major roles in this regard. The most important changes were observed in the RAS oncogene family (5).

The RAS family is formed of three members namely the H, K and N members. These three genes produce a type of protein in the inner surface of the cell membrane that has GTPase activity and may contribute to the transmission of signals. Animal studies indicated the role of RAS mutations in the development of lung cancer while human studies indicated the role of this gene in the development of the disease. Mutation of this gene normally occurs exclusively in Adenocarcinoma of the lung and is observed in 30% of the cases. It is worth mentioning that in non-smoking patients no mutation of the gene is observed. It seems mutation of K-ras is an independent prognosis factor. Other molecular disorders with less significance include c-myc and c-raf among oncogenes and retinoblastoma (Rb) and p53 among tumor suppressors (6).

The myc family plays a more significant role in small cell carcinoma. The C-myc form is mostly seen in the recurrent types. L-myc and N-myc are other members of the family. C-raf, c-erb-bl, c-fms, retinoblastoma gene, p53 gene are also among the suspected genes of this family (7).

P63 (which is also known as P73L, P51, P40 or KET) is a cloned gene of the 3q27-28 chromosome and is similar to the transcription factors of the P53 family regarding its structure and performance (8-13).

Accordingly, P63 can connect to certain parts of DNA, activate transcription of relevant promoters, and causes cell death if it occurs excessively. Unlike P53, the P63 gene codes at least 6 transcriptions in 2 different promoters through three C-terminal splicing modalities (12-13).

The 6 major products have variable capabilities including apoptosis and act as dominant-negative factors (14-18).

Although there is no evidence in support of the performance of P63 as the suppressor gene of the Knudson classic tumor. Recent investigations revealed that this gene is involved in embryonic development and cell differentiation through preservation of stem cells and storage of cell populations in certain human tissues (19-30).

Moreover, it was found out that the P63 protein can inhibit the growth of tumoral cells and lead to their death (25, 31).

Mutations of the P63 gene in human cancers are rare whereas reinforcement or excessive demonstration of these mutations are reported in some cancers such as head and neck cancer, nasopharynx, bladder cancer, oral cavity cancer, cervical cancer, and skin cancer (32-39). Mutations of P63 are rare in lung cancers whereas reinforcement or excessive demonstrations of them are seen in squamous cell carcinoma (SCC) and Adenocarcinoma (20, 38, and 39).

As seen, the aforementioned studies stress the importance of expression of P63 in the tissue samples of different types of lung cancer. However, a review of the existing information resources indicated that apparently no study has examined the status of this indicator in the sputum samples of patients with non-small cell lung cancer. Evidently, if acceptable results are obtained on this topic, many diagnostic problems in this regard are solved. The reason is that sputum samples are easily and quickly assessable and therefore this method considerably reduces the cost and time required for diagnosis. The aim of this research is to study the expression of P63 in the sputum samples of non-small cell lung cancer patients.

Materials and Methods

In a cross-sectional descriptive analytical study which was carried out in the Pathology Department of Tabriz University of Medical Sciences on patients with lung cancer, the expression of P63 in the sputum samples of patients with non-small cell lung cancer was investigated.

A total of 50 sputum samples were obtained from patients diagnosed with non-small cell lung cancer and 50 sputum samples were obtained from healthy individuals with the same age and gender. The samples were compared for the expression of P63 through immunohistochemistry.

The study population included 50 sputum samples of patients who were diagnosed with non-small cell lung cancer and visited the lung clinics of the Imam Reza training and treatment center and Sheikh Al-Raees clinic of Tabriz after approval of the project. 50 sputum samples were obtained from healthy individuals and were put in the control group.

In order to determine the expression of P63, the monoclonal kit for the P63 antibody (NCL-P63) (made in Novocastra) was used.

Statistical analysis

The collected data were analyzed by SPSS-17 statistical software. The collected data were expressed as percentage and mean ±

SD. Continuous (quantitative) variables were compared by Independent samples, Paired T test and ANOVA test. Categorical (qualitative) variables were compared by contingency tables and Chi-square test or Fisher's exact test. P-value ≤0.05 was considered statistically significant.

Ethical Considerations

All of the project expenses were paid by the project executive and patients were charged for none of the examinations. Patient information will also remain confidential. Since the study was not interventional, the oral consent of the participants was enough for including them in the study.

Results and Discussion

In this study, the expression of P63 in the sputum samples of 50 patients with non-small cell lung cancer and 50 healthy individuals was examined and compared. The following results were also obtained:
70% of the non-small cell lung cancer patients and 72% of the patients in the control group were male (P=0.826).

The mean age of non-small cell lung cancer patients and members of the control group was 63.20 ± 15.01 and 62.04 ± 12.50 years, respectively (P=0.676).

The mean expression of P63 in the non-small cell lung cancer patients and control group participants was 32.40 ± 19.98 and 2.48 ± 4.09% percent, respectively.

The mean expression of P63 in non-small cell lung cancer patients was significantly higher than the control group (P<0.001).

Mean of P63 expression in patients with lung SCC was 39.80 ± 19.39 percent, in patients with Adenocarcinoma was 25 ± 17.79 percent, in patients with Carcinoid atipic was 18 ± 12.54 percent and in patient with undifferentiated carcinoma was %60. Mean expression of P63 was significantly higher in patients with lung SCC (P=0.010). Results of the parametric analysis under study are shown in Table 1 based on tumor type and degree of differentiation.

The range of expression of P63 among patients in the two groups is depicted in Figure 1. The range of expression of P63 among patients with non-small cell lung cancer is shown in Figure 2 based on the type of lung tumor. The range of expression of P63 among patients with non-small cell lung cancer is shown in Figure 3 based on the degree of differentiation of lung tumors.

Recent studies have valued double smearing immunohistochemistry of P63 on de-colored slices and lung squamous cell carcinoma (SCC) cytology in the first month after H&E smearing (40-42).

Hamed et al. carried out a study on 71 cases of prostate biopsy with P63/AMACR in the first month of storing the smears of 10 samples. These researchers observed no considerable reduction in tonality. Of 63 of the samples, which were stored for 7-11 months for the examination of p63, 18 samples (30%) showed a lack of tonality, 32 cases (52%) showed reduced tonality, and 11 cases (18%) showed a tonality similar to P<0.0001. In this period, for the AMACR marker 7% of negativity, 3% of reduced smearing, and 90% of intensity were observed (p<0.05) (43).

Epstein and Dardik carried out a study on 105 prostate samples within 18 months using a cytokeratin marker with a high molecular weight. 59% of the samples showed optimal tonality after decolorization of the H&E smears. 13% of the samples were ambiguous and 9% of the samples

demonstrated tissue loss. 19% of the samples also remained colorless (44).

In another study that was carried out by Shtibans et al., the p63 marker was used on decolorized bronchoscopic cytology samples. Of the 10 squamous cell carcinoma (SCC) samples, 6 showed lack of tonality and 4 showed positive tonality. All of the Adenocarcinoma (12 cases), large cell carcinoma (4 cases) and metastatic Adenocarcinoma results were also negative (41).

Considering the degree of positivity of the p63 marker for the squamous cell carcinoma (SCC) tumor of lungs and negativity of this marker in lung cancer and SLC, this marker is significantly important (45-46). These findings prove the ability of this marker to differentiate the two tumors (46).

In a study that was conducted by Massion et al.(2004), 217 non-small cell lung cancer samples were studied for the expression of P63. The researchers stated that the expression of P63 in squamous cell carcinoma (SCC), large cell carcinoma and Adenocarcinoma was 88%, 42% and 11%, respectively (47).

Yang et al. (1999), carried out a study the results of which showed that expression of P63 in 52% of patients with invasive lung squamous cell carcinoma (SCC) is positive (48).

Wu et al., conducted a study in 2005 in which they used the expression of P63 to differentiate SLC from non-small cell lung cancer and stated that this marker can be used to differentiate the two tumors (49).

Au et al. (2004) studied the tissue samples of 284 non-small cell lung cancer patients for the expression of a number of immune indicators such as P63 through immunohistochemistry. The results showed that the expression of P63 in the squamous cell carcinoma (SCC) subtype is significantly higher than other subtypes (50). In the present study, the mean expression of P63 in patients with lung squamous cell carcinoma (SCC) was 39.80 ± 19.39 percent.

Au et al. (2004) examined the expression of P63 in 408 lung cancer samples using the immunohistochemistry method and reported that 97% of the squamous cell carcinoma (SCC) samples showed P63. Moreover, the expression of P63 in Adenocarcinoma and SLC was 30% and 37%, respectively (51).
Pelosi et al. (2002) examined the P63 immunoreactivity of 221 cases with class I non-small cell lung cancer and stated that the P63 immunoreactivity existed in 93.4% of squamous cell carcinoma (SCC) patients, 15.8% of Adenocarcinoma patients, 100% of adenosquamous carcinoma cases, 66.66% of large cell carcinoma patients and 2.7% of patients with Carcinoid tumor. They finally concluded that although P63 may be related to the pathogenesis of squamous cell carcinoma (SCC), the effect of prognosis is not known in this regard (52).

In a study that was conducted by Xu et al. (2014) in the pathology department of Zhengzhou University of China, the expression of P63 in lung tumors was studied and it was reported that the expression of P63 is higher in pulmonary squamous cell carcinoma (SCC) tumors than other tumors (53).

Table.1 Evaluation Age and expression of P63 in patients

		Age	P63
Tumor type	SCC	61.40 ± 15.08	39.80 ± 19.39
	Adenocarcinoma	63.74 ± 16.02	25.00 ± 17.80
	Carcinoid atipic	73.20 ± 6.22	18.00 ± 12.55
	Undifferentiated Carcinoma	48.00	60.00
Differentiation	Undifferentiated	61.00 ± 18.38	65.00 ± 7.07
	Poorly Differentiated	80.00 ± 0.00	5.00 ± 0.00
	Moderately Differentiated	60.73 ± 15.55	25.45 ± 16.47
	Well Differentiated	64.25 ± 14.59	38.33 ± 18.98

Figure.1 Distribution of P63 expression between two groups

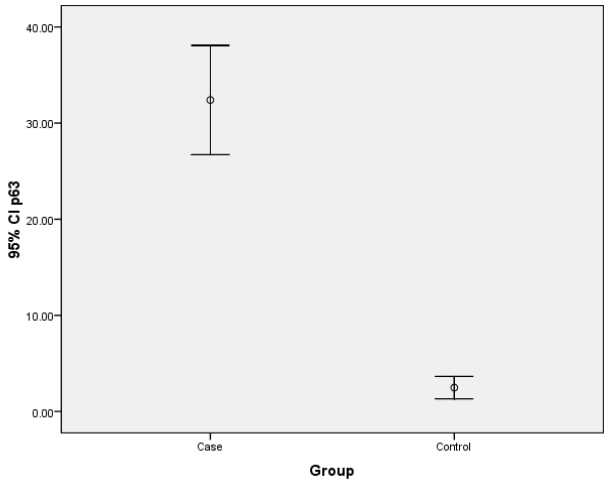


Figure.2 Distribution of P63 expression based on tumor pathology

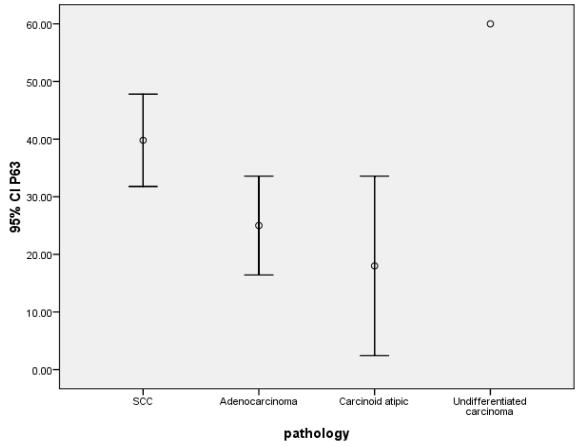
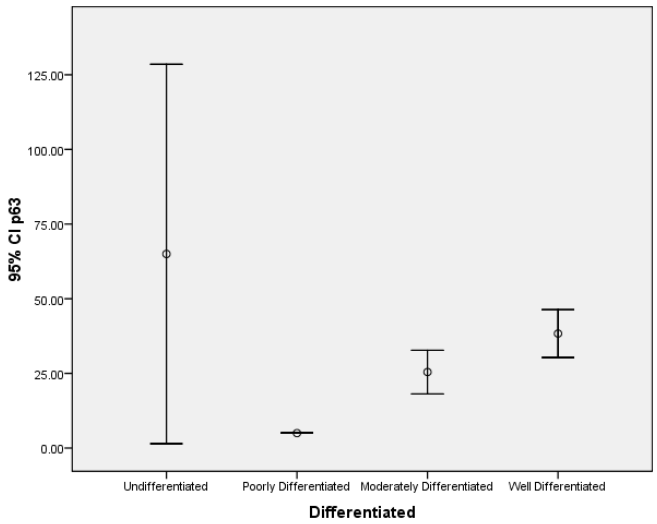


Figure.3 Distribution of P63 expression based on tumor Differentiated



Kargi et al. (2007) conducted a study in the pathology department of the Dokuz-Eylul University in Izmir, Turkey. These researchers studied the P63 marker in lung tumors and concluded that most of the patients with lung squamous cell carcinoma (SCC) have the P63 tumor marker, which is highly prevalent among patients with lung squamous cell carcinoma (SCC) (54).

In a study by Sethi et al. (2012) in the pathology department of Wayne University of Detroit (USA) the expression of P63 in lung cancer patients was examined and it was stated that all of the 20 patients with lung squamous cell carcinoma (SCC) had the P63 tumor marker (55).

In our study, the expression of P63 was studied in 27 patients with squamous cell carcinoma (SCC) and 25 patients showed an expression rate of more than 20%.

In 2011, Mukhopadhyay et al. conducted a study in the pathology department of New York University (USA) to examine the expression of P63 in non-small cell lung cancer patients. The researchers reported that the expression of P63 in patients with lung squamous cell carcinoma (SCC) is more than patients with lung Adenocarcinoma (56).

Similar to the above mentioned research, in the present study the mean expression of P63 in patients with lung squamous cell carcinoma (SCC) was significantly higher than other patients with non-small cell lung cancer (P=0.010). Sinna et al. (2011) carried out a study in the pathology department of New York University (USA) to study the expression of P63 in non-small cell lung cancer patients. The researchers reported that the P63 tumor marker demonstrates a high sensitivity and specificity for lung squamous cell carcinoma (SCC) cases (57).

Nober et al. (2013) performed a study in the pathology department of the Porto University of Portugal and examined the incidence of P63 in non-small cell lung cancer patients. The researchers stated that the P63 marker is a good diagnostic criterion for differentiating lung squamous cell carcinoma (SCC) from other tumors (58).

Conclusion

In this study, 50 patients with non-small cell lung cancer were selected and the expression of P63 in the sputum samples of these patients and another 50 healthy individuals was examined and compared.

The mean age of non-small cell lung cancer patients and participants in the control group was 63.20 ± 15.01 and 62.04 ± 12.50 years, respectively (P=0.676). The mean expression of P63 in the non-small cell lung cancer and control groups was 32.40 ± 19.98 and 2.48 ± 4.09 percent, respectively. The expression of P63 in non-small cell lung cancer patients was significantly higher than the control group members (P<0.001).

The mean expression of P63 in patients with lung squamous cell carcinoma (SCC), lung Adenocarcinoma, atypical Carcinoid, and undifferentiated carcinoma was 39.80 ± 19.39 percent, 25 ± 17.79 percent, 18 ± 12.54 percent, and 60%, respectively. The mean expression of P63 in patients with lung squamous cell carcinoma (SCC) was significantly higher (P=0.010). The mean expression of P63 in patients with a well-differentiated lung tumor was significantly higher (P=0.018).

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