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ASSESSMENT OF P63 IMMUNOHISTOCHEMICAL EXPRESSION IN LUNG CARCINOMA IN BANGLADESHI PATIENTS

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ABSTRACT

Background: Carcinoma of the lung is one of the major causes of cancer death. Lung tumors are histologically heterogeneous tumors with more than one histological type. The 2015 WHO classification recognizes four major histologic subtypes: squamous Cell Carcinoma (SCC), adenocarcinoma (ADC), large Cell Carcinoma and small Cell Carcinoma. Due to the recent advances in targeted therapies, subclassification of lung cancer has become increasingly important. p63 antibody panel can serve as a useful tool for subtyping of lung carcinoma in the bronchoscopic biopsy. **Objectives:** The purpose of this study was designed to determine the efficacy and role of p63 marker for the diagnosis of lung carcinoma. **Methodology:** This cross-sectional observational study was conducted in the Department of Pathology, Chittagong Medical College, Chattogram from March 2018 to February 2020. In this study, fifty-one biopsy samples of lung carcinoma were collected, processed, stained with hematoxylin, and eosin. Immunostaining for p63 was done in formalin-fixed paraffin-embedded tissue. All data were recorded in a pre-designed data sheet. Statistical analyses were carried out by using SPSS version 23 for Windows. A descriptive analysis was performed for all data. Observations were indicated by frequencies and percentages. Statistical significance was set at a "p" value<0.05. **Result:** In this study, The mean (±SD) age of the patients was 59.73 (±9.71) years with the minimum and maximum age of the patient were 38 years and 75 years respectively. A male predominance was observed in the series (86.3%). With regards to histological classification, 72.5% of patients had identified squamous cell carcinoma, 23.5% of patients had identified adenocarcinoma and only 2.0% of patients had identified as adenosquamous carcinoma and another 2.0% had small cell carcinoma. SCCs were more prevalent among males than females. In this study, 74.5% of patients were positive and 25.5% of patients were negative by p63 expression. Among the 37 squamous cell carcinoma patients, 81.1% of patients were positive and 18.9% of patients were negative by p63 expression. On the other hand, among the 14 patients, 57.1% of patients were positive and 42.9% of patients were negative by p63 expression in other than squamous cell carcinoma. There were higher sensitivity and positive predictive value. i.e. p63 expression could properly identify the squamous cell carcinoma. Moreover, due to the very limited number of adenosquamous carcinoma & small cell carcinoma, it was not possible to comment on the utility of p63 in such carcinoma. **Conclusion:** The study findings demonstrated p63 was found as a highly sensitive marker for squamous cell carcinoma. So, this panel p63 should be considered when confronted with a poorly differentiated pulmonary neoplasm of uncertain phenotype on bronchoscopic biopsy. A large scale prospective study with standardized techniques is desirable to validate the findings of the present study.

KEYWORDS: Lung tumors, adenocarcinoma, cell Carcinoma, bronchoscopic biopsy.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death both in men and women worldwide.[1] It is one of the most common cancer in the world. In 2012, there were an estimated 1.8 million new cases and 1.6 million deaths.[2] In 2018, lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths) worldwide.[3]

Histological subtypes of lung cancer that are morphologically recognizable on biopsy specimens

usually require further evaluation for the final diagnosis (Travis et al. 2011). It was previously believed that only biopsy material was appropriate for the distinction of lung carcinoma.[4] But, nowadays, the judicious use of immunohistochemistry assists in accurate histological categorization.[5]

p63 is a p53 homologous nuclear protein, which is expressed in basal cells of stratified squamous and glandular epithelia. In the lung, it has been mainly studied in different histologic subtypes of epithelial neoplasms, with the highest expression consistently noted in squamous cell carcinomas. The frequency of expression in pulmonary adenocarcinomas is lower, with most cases showing only focal staining.[6] The different isoforms of p63 are thought to have different functions as well. The truncated forms are thought to inhibit cell cycle arrest and apoptosis driven by transactivating p63/ p53 interaction.[7] The truncated isoforms are preferentially expressed in the basal cell compartment of normal epithelium and transactivating forms are more widely distributed in the benign and neoplastic epithelium (Signoretti et al. 2000).[8]

Consequently, different isoforms of p63 appear to play a role in maintaining the epithelial stem cell population, spurring epithelial differentiation, and inducing neoplasia.[9] In the literature, the sensitivity of p63 for the detection of pulmonary SCC in biopsy material reached up to 100% but the specificity of p63 was 88%.[10]

Several studies have attempted to understand the mechanism of action of the protease inhibitor and tumor suppressor genes that play a remarkable role in lung tumor pathogenesis.[11] p63 is a member of the p53 tumor suppressor gene family, and its effect on p53 can be agonistic or antagonistic.[12]

Abnormal expression of p63 has been identified in the oral cavity, skin SCCs, premalignant and invasive squamous lesions of the cervix, esophagus SCCs, urothelial carcinomas, and other tumors.

p63 has been reported to be highly positive in lung SCCs. Its expression rate is lower in other carcinomas but it can be useful in the differential diagnosis.[13]

OBJECTIVE

General Objective

To assess the p63 immunohistochemical expression in Lung Carcinoma in Bangladeshi patients.

Specific Objectives

1) To study the different histological subtypes of lung carcinoma.
2) To evaluate the expression of p63 in lung carcinoma.
3) To calculate the sensitivity, specificity, positive predictive value, and negative predictive value of p63 immunostaining.

MATERIAL AND METHODS

Type of the study: Cross-sectional observational study.
Place of the study: Department of Pathology, Chittagong Medical College and Care Investigation, Chittagong (For Immunohistochemical study).
Study period: March 2018 to February 2020.
Study population: The clinically suspected cases of lung carcinoma in the Chittagong Medical College, Chattogram during a specified time duration comprised the study population.
Study sample: Histopathologically diagnosed cases of lung carcinoma in the Department of Pathology, Chittagong Medical College, Chattogram during a specified time duration.
Sample size: 51 patients were included in the study as a sample.
Sampling technique: Consecutive sampling.

Eligibility criteria

Inclusion criteria

1. Patients of histopathologically diagnosed cases of primary lung carcinomas.
2. Patients who were willing to include himself in the research work.

Exclusion criteria

1. Patients having metastatic lung carcinoma.
2. Patients who refused to give consent.
3. Those who had received chemotherapy or radiation therapy for lung carcinoma.
4. Inadequate tissue in the block.

Data processing and analysis

McNemar test was applied for comparison of the marker p63 expression with histopathological examination. Validity tests (Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value & Accuracy) with 95% confidence interval were calculated for the two markers based on the pathological diagnosis. A,, p‟‟ value <0.05 was considered as statistically significant because all analyses were considered as a 95% confidence level. The SPSS software, version 23.0 was used for data analysis.

Procedure of data collection

It was a cross-sectional observational study in which the bronchoscopic biopsy material had been taken after getting permission from the Institutional Review Board. Then, data was recorded after taking properly informed written consent from the patient (Appendix-II) in the Department of Pathology, Chittagong Medical College from June 2018 to August 2019. Total of 51 cases of histopathologically diagnosed as lung carcinoma were selected for this study.

Statistical analysis

Data were entered into an Excel worksheet to generate a master sheet (Appendix X). After compilation, the data was presented in the form of tables by meticulous checking and rechecking. Statistical analysis of the results was done by using computer-based statistical

software, SPSS 23.0.0 version (SPSS Inc, Chicago, IL, USA). Results were shown as the table and expressed as frequency & percentage for qualitative data and mean ± SD for quantitative data and the McNemar test was applied for comparison of the marker p63 expression with histopathological examination. Validity tests (Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value & Accuracy) with 95% confidence interval were calculated for the two markers on the basis of pathological diagnosis. A „p" value <0.05 was considered as statistically significant because all analyses were considered as 95% confidence level.

RESULTS

Socio-demographic variables

Among 51 patients, most of the patients in this study were in the age group between 51 to 60 years (n=22, 43.1%) followed by age group between 61-70years (n=15, 29.4%). The mean (±SD) age of the patients was 59.73 (±9.71) years with a range of 38 and 75 years. About 86.3% (n=44) patients were male and 13.7% (n=7) patients were female. Among the patients, 52.9% were from low socioeconomic status and 47.1% of patients were from middle socioeconomic status. No high socioeconomic groups were found in this study.

Table 1: Distribution of the patients according to socio-demographic variables (n=51).

Socio-demographic variables	Frequency	Percent
Age (Years)		
≤40	2	3.9
41-50	7	13.7
51-60	22	43.1
61-70	15	29.4
≥ 71	5	9.8
Total	**51**	**100.0**
Mean ± SD	**59.73 ± 9.17**	
Age range(Min-Max)	**38-75**	
Sex		
Male	44	86.3
Female	7	13.7
Total	**51**	**100.0**
Socioeconomic condition		
Lower	27	52.9
Middle	24	47.1
High	00	00
Total	**51**	**100.0**

Smoking History

Out of 51 patients, most (n=42, 82.4%) of the patients had a history of smoking. Among smoker majority (40.5%) of them reported smoking 2 pack per day.

Table 2: Distribution of the patients according to smoking history (n=51).

Smoking history	Frequency	Percent
Yes	42	82.4
No	9	17.6
Total	**51**	**100.0**
Smoking per day among the smoker (n=42)		
1 pack per day	15	35.7
2 pack per day	17	40.5
3 pack per day	10	23.8
Total	**42**	**100.0**

Previous history of lung disease

Figure 1: Distribution of the patients according to the past history of lung disease. (n=51)

Among the 51 patients, (n=5, 9.8%) had a history of tuberculosis, and (n=3, 5.9%) had a history of COPD. Most of the patients (n=43, 84.3%) had no history of previous lung diseases.

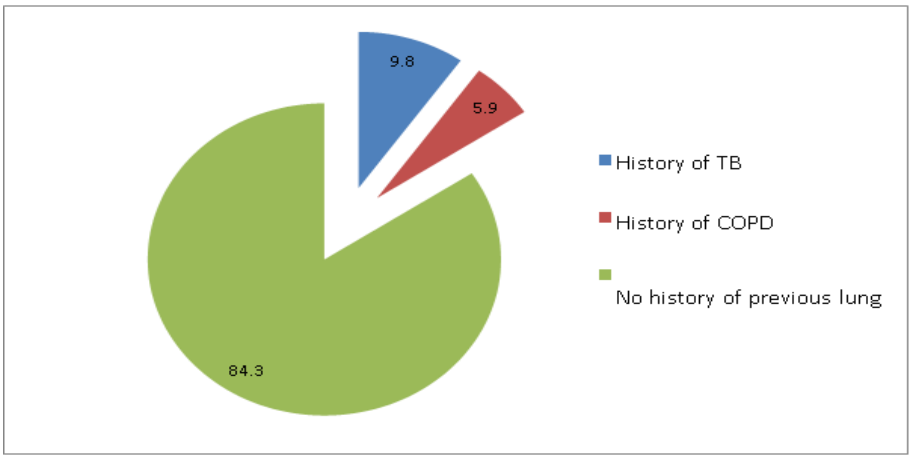

Figure 1. Pie chart of distribution of the patients according to the past history of lung disease.

Morphological types of tumor

Among 51 patients, the majority (37, 72.5%) of patients were squamous cell carcinoma followed by adenocarcinoma (12,23.5%). Only 1 (2%) case was adenosquamous carcinoma and another 1 (2%) case was small cell carcinoma by histopathological examination. It is shown in the following pie chart.

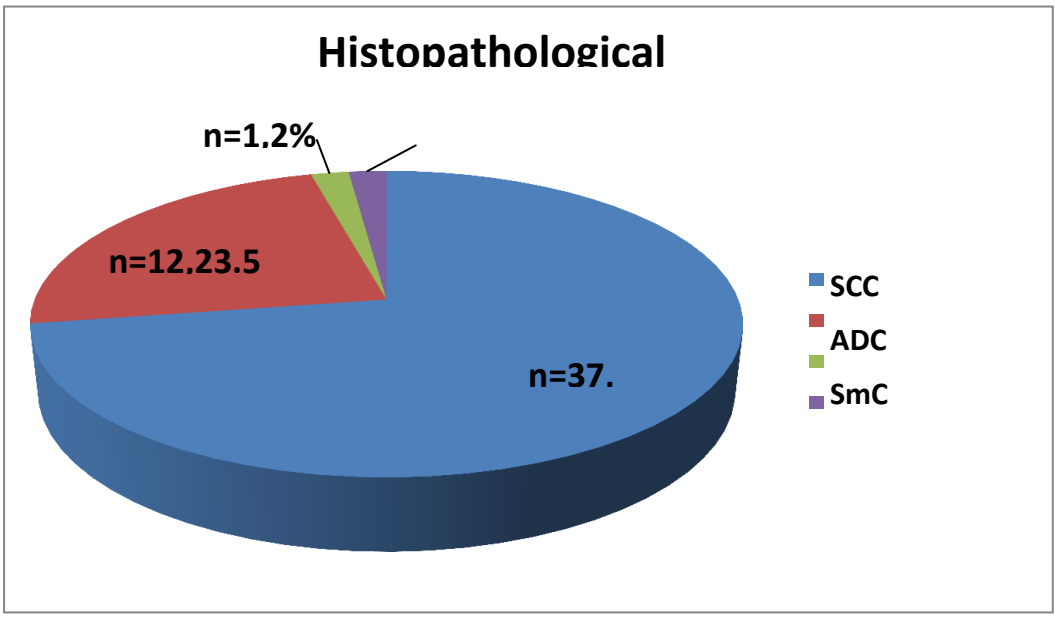

Figure 2: Pie chart of distribution of the patients according to histopathological classification (n=51)

Table 3: Distribution of the patients according to p63 expression (n=51)

	Frequency	Percent
Positive	38	74.5
Negative	13	25.5
Total	**51**	**100.0**

Table 3 shows the distribution of the patients according to p63 expression. Among the patients, three fourth (74.5%) of patients were positive and one fourth (25.5%) of patients were negative by p63 expression.

Table 4: p63 positive in squamous cell carcinoma (n=51).

	Histological types		
p63 expression	**Positive (Squamous cell carcinoma)**	**Negative (Other than SCC)**	**p-value***
Positive	30 (81.1)	8 (57.1)	
			0.999
Negative	7 (18.9)	6 (42.9)	
Total	**37 (100.0)**	**14 (100.0)**	

*McNemar test was done to measure the level of significance. The figure within parenthesis indicates in percentage.

Table 4 shows the distribution of the patients according to the positivity of p63 expression in squamous cell carcinoma. Among the 37 squamous cell carcinoma patients, (n=30) 81.1% of patients were positive and 18.9% of patients were negative by p63 expression. On the other hand, among the 14 patients not diagnosed as squamous cell carcinoma, 57.1% of patients were positive and 42.9% of patients were negative by p63 expression. There was no statistically significant (p>0.05) difference observed between histopathological examination and p63 expression i.e. these two tests (histopathological classification and p63 expression) did not differ for the detection of squamous cell carcinoma.

Table 5: Validity test for identifying squamous cell carcinoma by positive p63 expression.

Validity test	Percentage	95% CI
Sensitivity	81.1	72.7-89.3
Specificity	42.9	20.8-64.6
PPV	78.9	70.8-86.9
NPV	46.2	22.3-69.5
Accuracy	70.6	58.5-82.5

PP V = Positive Predictive Value NPV = Negative Predictive Value CI = Confidence Interval

Table 5 shows the validity test for identifying squamous cell carcinoma by the positivity of p63 expression. The sensitivity (95% CI) was 81.1% (72.7-89.3), specificity (95% CI) was 42.9% (20.8-64.6), positive predictive value (95% CI) was 78.9% (70.8-86.9), negative predictive value (95% CI) was 46.2% (22.3-69.5) and accuracy (95% CI) was 70.6% (58.5-82.5). There were higher sensitivity and positive predictive value. i.e. p63 expression could properly identify the squamous cell carcinoma.

Table 6: p63 negative in adenocarcinoma (n=51).

Adenocarcinoma			
	Positive	**Negative**	**p-value***
Negative	4 (33.3)	9 (23.1)	0.999
Positive	8 (66.7)	30 (76.9)	
Total	**12 (100.0)**	**39 (100.0)**	

*McNemar test was done to measure the level of significance. The figure within parenthesis indicates in percentage. Table 4.10 shows the distribution of the patients according to the negativity of p63 expression in adenocarcinoma. Among the 12 patients having adenocarcinoma, 33.3% of patients were negative and 66.7% of patients were positive by p63 expression. On the other hand, among the 39 patients not having adenocarcinoma, 23.1% of patients were negative and 76.9% of patients were positive by p63 expression. There was no statistically significant (p>0.05) difference observed between histopathological examination and p63 expression i.e. these two tests (histopathological examination and p63 expression) did not differ for the identification of adenocarcinoma.

Validity test to identify adenocarcinoma by p63 expression.

Table 7: Validity test for identifying adenocarcinoma by the negativity of p63 expression.

Validity test	Percentage	95% CI
Sensitivity	33.3	21.0-43.4
Specificity	76.9	70.4-85.0
PPV	30.8	11.1-55.0
NPV	78.9	72.2-87.2
Accuracy	66.7	56.6-79.0

PPV = Positive Predictive Value NPV = Negative Predictive Value C I = Confidence Interval.

Table 7 shows the validity test for identifying adenocarcinoma by negativity of p63 expression. The sensitivity (95% CI) was 33.3% (21.0-43.4), specificity (95% CI) was 76.9% (70.4-85.0), positive predictive value (95% CI) was 30.8% (11.1-55.0), negative predictive value (95% CI) was 78.9% (72.2-87.2) and accuracy (95% CI) was 66.7% (56.6-79.0). There were higher values of specificity and negative predictive value.

DISCUSSION

In the current study, 51 patients with lung carcinoma were included and most of them were between 51 to 70 years of age. The mean (±SD) age of the patients was 59.73 (±9.71) years with the minimum and maximum age of the patient were 38 years and 75 years respectively (Table 4.1). Jafarian et al. (2017) in their study reported that the mean age of the patients was 60 years; ranging from 35 to 81.[14] Zhou et al. (2017) in their study on adenocarcinoma patients reported the mean age of 60.7 years with a range from 24 to 85 years.[15] Oktay et al. (2018) in their study showed that the median age was 62 years.[16] These age differences may be due to different places of study.

Among the patients, 86.3% were male, and 13. 7% were female. The male & female ratio was 6.29. Oktay et al. (2018) in their study of 200 patients reported that 78.5% were male and 21.5% were female patients.[16] The higher incidence of lung carcinoma in the male may be due to personal habits such as smoking and more exposure to toxic agents.

The current study was conducted in a government-run tertiary care hospital of Bangladesh and it was observed that 52.9% of patients were from lower socioeconomic conditions and 47.1% of patients were middle socioeconomic conditions.

Smoking is one of the important risk factors for lung cancer. The observed patterns in lung cancer rates reflect the historical prevalence and variation in the trends of smoking among men and women. Incidence of the previously predominant squamous cell variety appears to be declining (although not universally), with a corresponding increase in adenocarcinoma (ADC)

variety in both genders (Varma et al. 2018). In the present study, most (82.4%) of the patients were smokers. In the present study distribution of the patients according to p63 expression, revealed 74.5% of patients were positive and 25.5% of patients were negative.

The utility of p63 for the identification of lung SCCs is well-known.[17] In the present study among the patients of squamous cell carcinoma, 81.1% of patients were positive and 18.9% of patients were negative by p63 expression. On the other hand, 57.1% of patients were positive and 42.9% of patients were negative by p63 expression in other than squamous cell carcinoma patients. There was no statistically significant ($p>0.05$) difference observed between histopathological examination and p63 expression i.e. these two tests (histopathological examination and p63 expression) did not differ for the identification of squamous cell carcinoma.

The sensitivity (95% CI) was 81.1% (72.7-89.3), specificity (95% CI) was 42.9% (20.8-64.6), positive predictive value (95% CI) was 78.9% (70.8-86.9), negative predictive value (95% CI) was 46.2% (22.3-69.5) and accuracy (95% CI) was 70.6% (58.5-82.5). There were higher sensitivity and positive predictive value. i.e. p63 expression could properly identify the squamous cell carcinoma.

Among the patients of adenocarcinoma, 33.3% of patients were negative and 66.7% of patients were positive by p63 expression. On the other hand, 23.1% of patients were negative and 76.9% of patients were positive by p63 expression in other than adenocarcinoma patients. There was no statistically significant ($p>0.05$) difference observed between histopathological examination and p63 expression i.e. these two tests (histopathological examination and p63 expression) did not differ for identification of adenocarcinoma.

The validity test for identifying adenocarcinoma by negativity of p63 expression was observed. The sensitivity (95% CI) was 33.3% (21.0-43.4), specificity (95% CI) was 76.9% (70.4-85.0), positive predictive value (95% CI) was 30.8% (11.1-55.0), negative predictive value (95% CI) was 78.9% (72.2-87.2) and accuracy (95% CI) was 66.7% (56.6-79.0). There were higher values of specificity and negative predictive value.

CONCLUSION

In conclusion, p63 was found as a highly sensitive marker for squamous cell carcinoma. The present study was intended to find out the role of commonly used IHC marker, p63 in the classification of lung carcinoma in the bronchoscopic biopsy. The study findings demonstrated that there was no statistically significant ($p>0.05$) difference observed between histopathological examination and expression of p63. So, morphological diagnosis is the gold standard technique for the categorization of a tumor. Majority of the lung carcinoma could be diagnosed by histopathological examination alone. In this study, it was observed the validity test for identifying squamous cell carcinoma and adenocarcinoma by p63 immunostaining.

REFERENCES

1. Jemal, A., Siegel, R., Xu, J., and Ward, E., (2010).„ Cancer statistics, 2010". *CA: a cancer journal for clinicians*, *60*(5): 277-300.
2. Ferlay, J., Colombet, M., Soerjomataram, I., Siegel, R., Torre, L. and Jemal, A., (2018).„ Global and regional estimates of the incidence and mortality for 38 cancers: GLOBOCAN 2018". Lyon: International Agency for Research on Cancer. *World Health Organization*, 394-424.
3. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A., (2018). „Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries". *CA: a cancer journal for clinicians*, *68*(6): 394-424.
4. Mukhopadhyay, S. and Katzenstein, A.L.A., (2011).„ Subclassification of non- small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6". *The American journal of surgical pathology*, *35*(1): 15-25.
5. Bhatti, V., Kwatra, K.S., Puri, S., and Calton, N., (2019).„ Histopathological spectrum and immunohistochemical profile of lung carcinomas: A 9-year study from a tertiary hospital in North India". *International Journal of Applied and Basic Medical Research*, *9*(3): 169.
6. Sheikh, HA., Fuhrer, K., Cieply, K., Yousem, S., (2004),, p63 expression in assessment of bronchioloalveolar proliferations of the lung", *Mod Pathol*, 17: 1134–40.
7. Yang A., Kaghad M., Wang Y., Gillett E., Fleming MD., Dotsh V., (1998),, p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death- inducing, and dominant-negative activities", *Mol Cell*, 2: 305–16.
8. Signoretti, S., Waltregny, D., Dilks, J., Isaac, B., Lin, D., Garrawy, L, 2000' p63 is a prostate basal cell marker and is required for prostate development", *Am J Pathol*, 157: 1769–75.
9. Nylander, K., Vojtesek, B., Nenutil, R., Lindqren, B., Roos, G., Zhanxiang, W., (2002),, Differential expression of p63 isoforms in normal tissues and neoplastic cells", *J Pathol*, 198: 417–27.
10. Wei, J., Zaika, E., and Zaika, A.,(2012)., p53 family: role of protein isoforms in human cancer". *Journal of nucleic acids*, *2012*.
11. Yaman, B., Nart, D., Ekren, P.K., Çok, G. and Veral, A., (2015)., Expression of p63, TTF–1 and Maspin in Non-Small Cell Lung Carcinoma and Their Effect on the Prognosis and Differential Diagnosis". *Turkish Journal of Pathology*, *31*(3):

163-174.

12. Wang, B.Y., Gil, J., Kaufman, D., Gan, L., Kohtz, D.S., and Burstein, D.E., (2002)., P63 in the pulmonary epithelium, pulmonary squamous neoplasms, and other pulmonary tumors‟. *Human pathology*, *33*(9): 921-926.
13. Afify, A.M., & Al-Khafaji, B.M., (2002),, Diagnostic utility of thyroid transcription factor-1 expression in adenocarcinomas presenting in serous fluids‟, *Acta Cytol*, 46: 675-8.
14. Jafarian, A.H., Gharib, M., Roshan, N.M., Sherafatnia, S., Omidi, A.A., and Bagheri, S., (2017).,,The diagnostic value of TTF-1, P63, HMWK, CK7, and CD56 immunostaining in the classification of lung Carcinoma‟. *Iranian Journal of pathology*, *12*(3): 195.
15. Zhou, C., Zhao, J., Shao, J., and Li, W., (2017).,, Prognostic relevance of TTF-1 expression in stage I adenocarcinoma‟. *Oncotarget*, *8*(64): 107462.
16. Oktay, E., Oflazoglu, U., Varol, Y., Tanriverdi, O., Mermur, N., Arda, H.U., et al. (2018).,, The prognostic role of thyroid transcription factor-1 in lung adenocarcinoma‟. *Journal of Cancer Research and Therapeutics*, *14*(12): 1201.
17. Argon, A., Nart, D. and Veral, A., (2015).,, The Value of Cytokeratin 5/6, p63, and Thyroid Transcription Factor-1 in Adenocarcinoma, Squamous Cell Carcinoma, and Non-Small-Cell Lung Cancer of the Lung‟. *Turkish Journal of Pathology*, *31*(2): 81-88.