Applying P40 and TTF-1 in the Subtyping of Poorly Differentiated Non-Small Cell Lung Carcinoma in Bronchoscopic Biopsy

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ABSTRACT:

BACKGROUND:

Lung cancer is the leading cause of cancer-related deaths worldwide, regardless of gender. It is categorized into two main groups: small cell lung carcinoma (SCLC, 15% of all lung cancers) and non-SCLC (NSCLC, 85% of all lung cancer). Increasing knowledge of the molecular pathology of lung cancers has led to their classification into specific subtypes according to appropriate treatments and molecular-targeted therapies.

OBJECTIVE:

Evaluation of the expression of P40 and TTF-1 in randomly selected samples from Iraqi patients who have NSCLC and correlate their expression with the clinicopathological variables.

PATIENTS AND METHODS:

This is a retrospective study of 40 patients who were diagnosed to have NSCLC. Formalin fixed, paraffin embedded tissue blocks.P40 and TTF-1 immunoreactivity and pattern of staining were evaluated in immunohistochemical stained slides and correlated the results with clinicopathological parameters.

RESULTS:

55% of the tumors were squamous cell carcinoma while 45% were adenocarcinoma. The sensitivity and specificity of P40 for the SQC diagnosis were 100% while the sensitivity of TTF-1 for ADC diagnosis was 88.9% and specificity was 100%.

CONCLUSION:

P40 and TTF-1 can be used as a reliable minimalistic panel for accurate subtyping of NSCLC.

KEYWORDS: thyroid transcription factor, P40 expression.

INTRODUCTION:

Lung cancer (LC) is the most commonly diagnosed cancer world-wide with a more than twelve million people newly diagnosed cases per year. This cancer is the leading cause of cancer-related death world-wide [(1)].An emerging issue of the so-called therapeutic pathology[(2)] in lung cancer regards the precise subtyping of non-small cell lung carcinomas (NSCLC),[(3)] which may yet present a difficult challenge by morphology alone when dealing with limited material and/or poorly differentiated tumors. The reproducibility rate of the pathologic diagnosis, based on routine light microscopic features are satisfactory for distinguishing small cell lung carcinoma (SCLC) from non-small cell lung carcinoma (NSCLC) and for detection of glandular versus squamous differentiation in well to moderately differentiated adenocarcinoma (ADC). However, it is less than satisfactory for identifying high grade NSCLC cases especially in small biopsy tissues obtained by bronchoscopy or tru-cut biopsy due to a variety of factors, such as crush artifact, necrosis, less than optimal fixation and the overlapping cytologic characteristics of these tumors [(4)].The current WHO classification of lung tumors differs from its predecessors in including not only criteria based on routine light microscopy but also immunophenotypic criteria based on a limited number of commonly available immunostains [(5)].

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The relative analysis considering the sensitivity and specificity of immunomarkers in diagnosis of lung carcinoma in small biopsy specimens is limited [6]. In this study, we focused on the immunohistochemical characterization of NSCLC in small biopsy samples using specific antibodies (p40 for SQC and TTF-1 for ADC) to discriminate between these two major histological subtypes and to find out their accuracy in such purposes.

AIM OF THE STUDY:

The aim of this study is to apply p40 and TTF-1 immunohistochemical markers on small lung biopsies to emphasize the diagnosis of squamous cell carcinoma and adenocarcinoma and to find out the sensitivity, specificity, positive predictive value and negative predictive value of these two immunomarkers in these diagnostic purposes.

PATIENTS AND METHODS:

- Formalin fixed, paraffin embedded tissue blocks were collected from archived materials from Ghazi Al-Hariri specialized surgical hospital in Baghdad (covering the period from March 2018 to May 2019)
- Formalin-fixed paraffin tissue blocks represent 40 cases of bronchogenic lung carcinoma, all of them were small bronchoscopic biopsies
- All patients were newly diagnosed and received no prior therapy.
- Information regarding the patient's age at presentation, gender and type of samples were obtained from patient's request forms.
- Age of the patients range 42 years to 84 years.
- Three sections of 5 µm thickness were taken from each block, the first was stained with hematoxylin and eosin stain (H&E) for histological revision, the others sections were stained immunohistochemically for TTF1 and P40.
- **Immunohistochemical staining procedure.**

Immunohistochemical expression of TTF-1 & P40 in non-small cell lung carcinoma cells should be **intense brown nuclear** staining and are better evaluated by the percentage of positive tumor cells as to classify the results as the following scoring system [7]:

- **0** : if staining was completely absent in the relevant cells.
- **Score 1** : focally positive (<10% positive cells).
- **Score 2** : , moderately positive (>10% to <50% positive cells)
- **Score 3**: intensely positive (more than 50% positive cells).

Score 0 and 1 considered as negative for the immunohistochemical marker expression, while score 2 and 3 considered as a positive expression.

Statistical Analysis:

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Independent t-test (two tailed) was used to compare the continuous variables among study groups accordingly. Pearson's Chi–square test was used to assess statistical association between different variables. A level of P – value less than 0.05 was considered significant.

RESULTS:

Study patients age was ranging from 42 to 84 years with a mean of 69.12 years and a standard deviation (SD) of ± 11.3 years. The highest proportion of study patients was aged ≥ 60 years (90%). Regarding gender, proportion of males was higher than females (75% versus 25%) with a male to female ratio of 3:1.

Regarding smoking, 70% of study patients were current smokers.

Table (1): Distribution of study cases by carcinoma type

Carcinoma type	No. (n= 40)	Percentage (%)
Squamous cell carcinoma	22	55.0
Adenocarcinoma	18	45.0

P40 expression score

Score (3) was presented in 50% of cases while score (0) was presented in 45%. All squamous cell carcinoma cases and 66.7% of males showed positive expression with a **significant association** ($P < 0.05$) between P40 expression and both of gender and carcinoma type.

No statistical significant associations ($P = 0.781$) between P40 expression and age as shown in Table(2).

Table (2): Association between P40 expression and certain characteristics

Variable	P40 expression		Total (%) n= 40	P - Value
	Score 2&3 (%) n= 22	Score 0 (%) n= 18		
Age (Year)				
< 60	2 (50.0)	2 (50.0)	4 (10.0)	**0.781**
60 - 69	9 (50.0)	9 (50.0)	18 (45.0)	
≥ 70	11 (61.1)	7 (38.9)	18 (45.0)	
Gender				
Male	20 (66.7)	10 (33.3)	30 (75.0)	**0.01**
Female	2 (20.0)	8 (80.0)	10 (25.0)	
Tumor type				
Squamous cell carcinoma	22 (100.0)	0 (0)	22 (55.0)	**0.001**
Adenocarcinoma	0 (0)	18 (100.0)	18 (45.0)	

TTF1 expression

We noticed that 55% of cases scored (0) and 30% of them scored (3). All squamous cell carcinoma cases and 73.3% of males showed negative expression with a **significant association** ($P < 0.05$) between TTf1 expression and both of gender and carcinoma type. **No statistical** significant associations ($P = 0.723$) between TTF1 expression and age as shown in Table(3).

Table (3): Association between TTF1 expression and certain characteristics

Variable	TTF1 expression		Total (%) n= 40	P - Value
	Score 2&3 (%) n= 16	Score 0 (%) n= 24		
Age (Year)				
< 60	2 (50.0)	2 (50.0)	4 (10.0)	**0.723**
60 - 69	8 (44.4)	10 (55.6)	18 (45.0)	
≥ 70	6 (33.3)	12 (66.7)	18 (45.0)	
Gender				
Male	8 (26.7)	22 (73.3)	30 (75.0)	**0.002**
Female	8 (80.0)	2 (20.0)	10 (25.0)	
Cancer type				
Squamous cell carcinoma	0 (0)	22 (100.0)	22 (55.0)	**0.001**
Adenocarcinoma	16 (88.9)	2 (11.1)	18 (45.0)	

Table(4): Sensitivity, specificity, and accuracy of P40 expression score result

P40 Expression	Cancer Type		
	Squamous CC	Adenocarcinoma	Total
Positive	22	0	22
Negative	0	18	18
Total	22	18	40

For squamous Cell Carcinoma, the sensitivity =100%, specificity= 100 and accuracy of P40 expression score was 100%. **+ve and –ve** predictive values were 100% as shown in Table (4).

Table (5): Sensitivity, specificity, and accuracy of TTF1 expression score

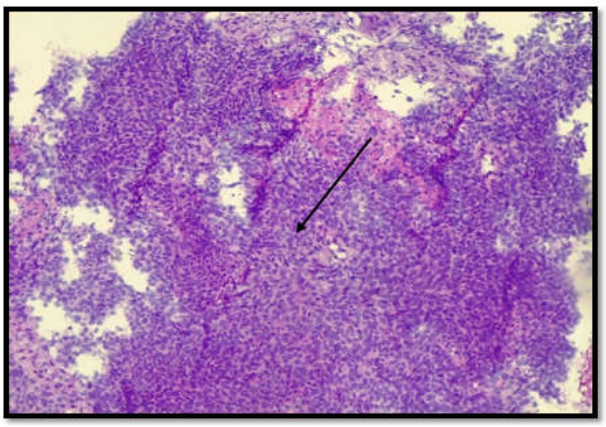
TTF1 Expression	Cancer Type		
	Adenocarcinoma	Squamous CC	Total
Positive	16	0	**16**
Negative	2	22	**24**
Total	18	22	**40**

For adenocarcinoma, the sensitivity = 88.9%, specificity = 100% and accuracy of TTF1 expression score was 95%. **+ve** predictive value was 100% while **–ve** predictive value was 91.7% as shown in table (5).

We noticed that all cases that showed **P40 + / TTF1 –** results were squamous CC, while all cases that showed **P40 - / TTF1 +** or showed **P40 - / TTF1 -** results were adenocarcinoma as shown in Table (6).

Table (6): Panel of P40 and TTF1 expression in relation to carcinoma type

P40 / TTF1 Expression	Cancer Type		
	Squamous CC	Adenocarcinoma	Total
$P40^{+}$ / $TTF1^{-}$	22 (100.0)	0 (0)	22
$P40^{-}$ / $TTF1^{+}$	0 (0)	16 (100.0)	16
$P40^{-}$ / $TTF1^{-}$	0 (0)	2 (100.0)	2
$P40^{+}$ / $TTF1^{+}$	0	0	0
Total	22	18	40



Figure(1) : poorly differentiated squamous cell carcinoma(arrow) (X10) (H&E).

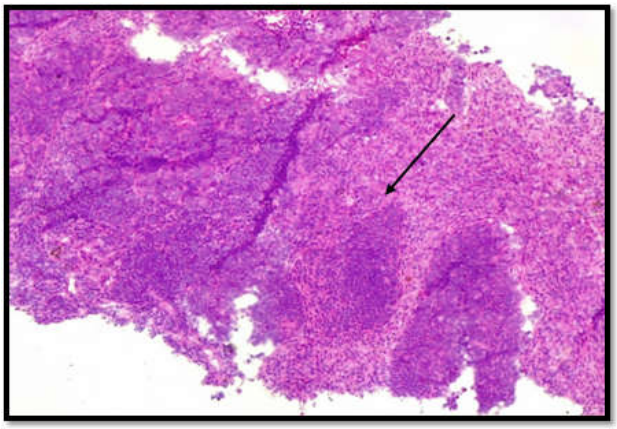
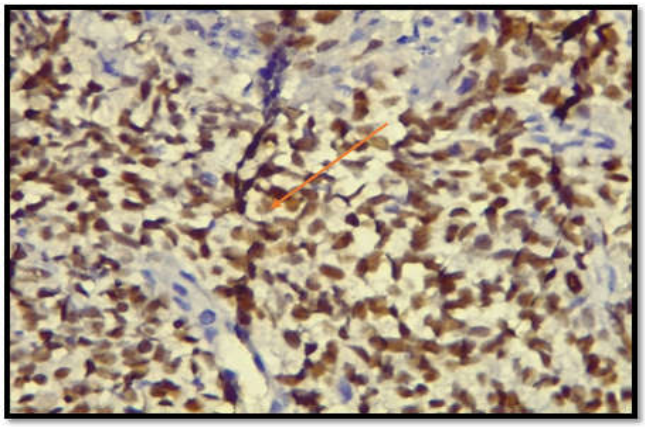
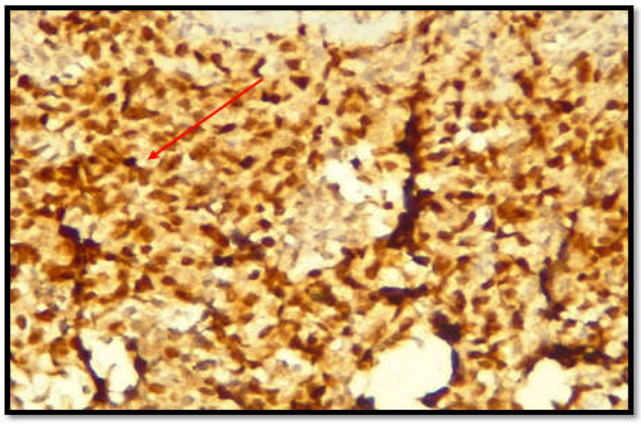


Figure (2) :poorly differentiated adenocarcinoma(arrow) (X10) (H&E).



Figure(3): strong nuclear reactivity for P40 (score 3) (arrow) in SQC case (40X)(Immunohistochemistry).



Figure(4): strong nuclear reactivity for TTF-1 (score 3) (arrow) in ADC case (40X)(Immunohistochemistry).

DISCUSSION:

P40 expression:

Regarding age, there was **no significant** statistical correlation with P40 expression and age which is correspond with **Affandi KA et al.(2018 [8]**and **Bishop JA et al.(2012)[9]** study results.

About the gender, there was a **significant association** between P40 expression and gender.

This result is similar to **Affandi KA et al. (2018) [8]** results that showed a significant association also, as do **Tatsumori et al (2014) [10]** in their study results.

Regarding tumor type, all squamous cell carcinoma showed positive expression for P40 with sensitivity of (100%) , while none of adenocarcinoma cases show positivity for P40 with (100%) specificity and **a significant** association . In the study of **Walia R et al (2017)[11]** who detected that all of the 54 cases of squamous cell carcinoma were positive for P40 and were negative in all 82 ADC cases. The 100% sensitivity and specificity documented for P40 in their study and this study results, support and strengthened the claim that '**P40 is the best currently available SQC marker'. [9, 11] Prabhakaran S et al 2019 [15]** also detected a significant association with

94.8% sensitivity and 100 % specificity, thus agree with the present study results.

TTF-1 expression:

Regarding age, there was **no significant** statistical correlation with TTF-1 expression and age. This result showed agreement with **Bhatti V.et al. (2019)** [12] study which also reflected a non-significant correlation between TTF-1 expression and age.

About the gender, there was a **significant** association between TTF-1 expression and gender.

Yang GY et al (2014) [13] study detected a **non-significant** statistical correlation between TTF-1 expression and gender which **differ** with this study result.

This discrepancy between both studies may be related to random patient's gender selection.

Regarding tumor type, the **sensitivity** of TTF-1 expression score for ADC =**88.9%, specificity = 100%.** with a **significant** association between TTF-1 expression and carcinoma type.

The present study results correspond with **F.R. Muhammad (Iraq 2015)**[14] study results which detected in her study on Iraqi patients that 17 out of 21 ADC cases showed positive expression of TTF-1 while all of the 65 SQC cases were negative with the specificity of 100% and 80.9% sensitivity.

Walia R et al (2017)[11]study demonstrated on their study on Indian patients an approaching results to this study with 70 out of 82 cases of ADC showed positive expression for TTF-1 and only positive in 1 out of 54 SQC with specificity 85.3% and 98.1% sensitivity, thus agree with this study results.

CONCLUSION:

1. The two- antibodies panel approach may contribute to accurate subtyping of NSCLC in small biopsies and a reduction in the NSCLC-NOS diagnostic categories.
2. P40 and TTF-1 form a practical panel for the distinction between pulmonary SQC and ADC in routine histopathology practice. This panel offered the best differentiation and correctly separated the two most common lung cancers with 100% specificity and 100% positive predictive value of both markers.
3. TTF-1 positive cases with P40 negativity are most likely to be ADC and the reverse is true for SQC as all SQCs in this study were TTF-1 negative.

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