ISSN (O): 2349-5332
CODEN: IRJPHY

IMMUNOHISTOCHEMICAL PROFILING OF BIOMARKERS IN LUNG CANCER CASES AND THEIR CORRELATION WITH CLINICAL AND DEMOGRAPHIC STATUS

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Submitted on: 07.09.2022; **Revised on:** 28.10.2022; **Accepted on:** 03.11.2022

Abstract:

Lung cancer is one of the prevalent cancers that impact the life of the patients. The main objectives of the present study were to profile lung cancers from immunohistochemical points of view and to correlate the immune profiling with demographic variables and clinical status of the disease. The methods of the present study included the conduction of retrospective study of patient's files with lung cancer. A total of 150 cases were selected. The results showed that males were more likely to have lung cancer, and the age of most patients was over 65 years. Most patients were smokers. The most prevalent tumors were adenocarcinoma, followed by squamous cell carcinoma, and neuroendocrine tumors. It can be concluded that the distribution of risk factors and lung tumor types followed the patterns of western studies. It is recommended to follow smoking cessation policies to lower the prevalence of lung cancer.

Keywords: Lung cancer, adenocarcinoma, squamous cell carcinoma, neuroendocrine tumors, smoking

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Indian Research Journal of Pharmacy and Science; 33(2022)2744-2754;
Journal Home Page: https://www.irjps.in

INTRODUCTION:

According to GLOBOCAN's predictions of its incidence and mortality in 2020, lung cancer will account for 11.4% of the total 19.3 million cases of cancer and will continue to be the leading cause of cancer-related deaths with 1.8 million fatalities[1]. The two types of lung cancer are small cell lung cancer, also known as SCC, which accounts for 80–85 percent of cases, and non-small cell lung cancer, also known as NSCLC (SCLC). Even though smoking is the primary factor in the development of lung cancer, up to forty percent of Asians and fifteen percent of Caucasians who have the disease are not smokers. In non-smokers, the factors that put them at risk and the origins of the illness are still largely unknown. In most cases, non-small cell lung cancer is not diagnosed until an advanced stage of the disease has been reached [2].

Cancer is the leading cause of death worldwide, and the non-small cell lung cancer (NSCLC) subtype of lung cancer, which accounts for 80% of all lung cancer subtypes, is the deadliest form of the disease. Patients who are diagnosed with locally advanced non-small cell lung cancer may have the option of undergoing immediate surgical intervention. Nevertheless, the overall survival rate after five years is only 59 percent. The extensive research that was done to determine whether adjuvant chemotherapy (ACT) could increase survival rates revealed a general benefit in survival rates at 5 years of 7%. Although the evaluation of recurrence risk and the subsequent need for ACT is only dependent on tumor stage, more than 25% of patients with stage IA/B cancers will experience a relapse during their course of treatment (TNM classification). Adjuvant targeted therapy has just been granted approval for the treatment of EGFR-mutated non-small cell lung cancer that has been surgically removed, and clinical trials are currently evaluating a variety of targeted therapies and immunotherapies in adjuvant settings. The need for improved patient selection is highlighted by factors such as costs, duration of treatment, the production of resistant clones, and side effects. Prognostic and theranostic markers need to be identified and validated before they can be used to perform a more accurate stratification of individuals who might benefit from adjuvant therapy. In this article, we provide a list of the most recent clinical, pathological, and molecular prognostic biomarkers that have been validated and shown to affect the outcomes of resection for NSCLC. In addition to this, we discuss molecular biomarkers that are currently being researched and have the potential to become useful tools for ACT in resected non-small cell lung cancer [3].

The very aggressive nature of lung cancer contributes to the high morbidity and mortality rates associated with the disease. The incidence of lung cancer is rising globally, but particularly in India. No longer are non-small cell lung

carcinoma and small cell lung carcinoma the only types of lung cancer recognized in today's medical community (NSCLC). The correct subtyping of poorly differentiated non-small cell lung cancer into adenocarcinoma and squamous cell carcinoma has a direct bearing on the care that patients receive as well as their prognosis. In light of this, research is being done on a large number of different compounds in order to develop more specific treatments. One of these biomarkers, known as epidermal growth factor receptor, is thought to be useful in the practice of targeted therapy for adenocarcinoma (EGFR). The incidence of adenocarcinoma was highest between the ages of 61 and 70, and the disease was more common in men. Adenocarcinoma made up 55% of non-small cell lung cancer. The EGFR gene was found to be expressed in 89% of the adenocarcinomas. Conclusions: Immunohistochemical markers have the ability to subtype poorly differentiated non-small cell carcinoma, which has direct implications for the treatment approaches that are currently being utilized [4].

Lung cancer is by far the most prevalent form of the disease that ultimately results in death. Cancer is responsible for the deaths of approximately one million people every year [5, 6]. The most common histologic type of lung cancer is called adenocarcinoma, and non-small cell lung carcinoma (also known as NSCLC) accounts for 80–85 percent of all lung carcinomas. Despite the improved treatment options that are currently available, the prognosis for these individuals continues to be dismal, with an overall survival rate of less than 15% after five years [7].

It is essential to diagnose and differentiate lung adenocarcinoma (LUAD) from lung squamous cell carcinoma (LUSC) in order to select the most appropriate method of treatment. This is because recent targeted medicines require precise subtyping of nonsmall-cell lung cancer (NSCLCs). At the present time, there are a number of biomarkers that could be used to differentiate between LUAD and LUSC; however, these biomarkers have lower levels of sensitivity, specificity, and clinical utility. LUSC had significantly higher levels of SPATS2 and CLCA2 expression than LUAD. When compared to LUSC, the expression of ST6GALNAC1 and Adipophilin was significantly higher in LUAD (P 0.001). When it comes to accurate subtyping and diagnosis, the CLCA2, SPATS2, ST6GALNAC1, and Adipophilin test have a sensitivity and specificity rating of 100% respectively. Only the survival rate of patients with negative and positive CLCA2 expression was found to significantly differ (P=0.038 and P=0.019, respectively). This was the only observation that showed a significant difference. Using a combination of the biomarkers CLCA2, SPATS2, ST6GALNAC1, and Adipophilin, it is possible to make a diagnosis of lung cancer that

has the highest possible sensitivity as well as the highest possible specificity [8].

Non-small cell lung cancers account for nearly 89 percent of all cases of lung malignancy (NSCLC). Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the two primary histological subtypes of lung cancer. Lung adenocarcinoma accounts for approximately 45 percent of lung cancer cases, while lung squamous cell carcinoma accounts for approximately 25 percent [9]. It is essential to make an accurate histopathological diagnosis and differentiate LUAD from LUSC in order to choose the best course of treatment. This is because recently developed targeted therapies call for exact sub-typing of NSCLCs in order to be effective [10]. It is difficult to make an accurate diagnosis based solely on the standard histopathological evaluation because small biopsies with few tumor cells and tumors with ambiguous structures brought on by poor differentiation or necrosis make it difficult to make a diagnosis. The molecular profiling and histopathological characteristics of LUAD and LUSC differ greatly from one another. As a direct result of this, immunohistochemistry is now highly recommended for use in clinical settings [9]. Even though they have a lower clinical relevance, sensitivity, and specificity, there are now a number of biomarkers that have been put through immunohistochemical testing and have been found to be helpful in differentiating LUAD from LUSC. These biomarkers have undergone evaluation [10].

There is a subset of pulmonary tumors known as neuroendocrine tumors of the lung. These tumors are distinguished by their unique morphofunctional characteristics. Under a light microscope, the four primary types of tumors (typical and atypical carcinoids, small cell lung cancer, and large cell neuroendocrine carcinoma) each exhibit a distinctive pattern of cell arrangement (organoid nesting, palisading, a trabecular pattern, and rosette-like structures), a variable number of mitoses, and either the presence or absence of necrosis. Necrosis is an accumulation of dead cells in the tissue that can be When observed through a microscope, neuroendocrine tumors are distinguished by clusters of cells that contain cytoplasmic granules, in particular the so-called dense-core neurosecretory granules. These granules are responsible for the tumor's ability to secrete hormones. Neuroendocrine cells have the capacity to secrete hormones either into the bloodstream or in a manner that is referred to as paracrine secretion. At the level of light microscopy, some pulmonary tumors do not exhibit neuroendocrine morphology; however, they do demonstrate ultrastructural and/or immunohistochemical traits that are characteristic of neuroendocrine differentiation [11].

The glycoprotein known as synaptophysin was first identified in neural tissue. It is localized in

the presynaptic vesicles of nearly all nerve cells. Synaptophysin was first identified in neural tissue. Wiedenmann and colleagues were the first to describe and give the protein its name in the neurons of the brain, spinal cord, retina, and neuromuscular junctions. They also came up with the name. [12, 13] It is a transmembrane protein that is an integral part of the membrane and has a molecular weight of 38 kilodaltons.

In cases of lung adenocarcinoma, the expression of the TTF-1 gene can serve as both a diagnostic marker and a reliable indicator of the patient's prognosis. This is because TTF-1 is a gene that encodes the thyroid transcription factor. However, as demonstrated by the positive correlation between TTF-1 expression and EGFR mutations, its good prognostic ability may be the result of epidermal growth factor receptor (EGFR) mutations that make the receptor more sensitive. The fact that TTF-1 expression has been found to have a positive correlation with EGFR mutations is evidence that this is the case. The authors investigated the prognostic significance of TTF-1 expression in patients who had lung adenocarcinoma. This was done in relation to the presence or absence of EGFR-sensitizing mutations in the patients [14].

For the purpose of this research, a total of 173 patients participated. There were 84 patients who were found to have EGFR-sensitizing mutations, which corresponds to a detection rate of 51.4%. It was discovered that there was a significant correlation between TTF-1 expression and EGFR-sensitizing mutations. TTF-1 expression was found to be positive in 139 patients, which represents 80.3% of the total ($p< 0.001$). Patients who were diagnosed with lung adenocarcinoma and who tested positive for the TTF-1 gene had a higher overall survival (OS) rate compared to patients who did not test positive for the gene (19.3 months versus 5.8 months, $p< 0.001$). Having a positive TTF-1, having Stage IV M1a, having a good performance status, and having EGFR-sensitizing mutations were all independently associated with a longer OS, as determined by the findings of a Cox regression analysis. TTF-1 positivity was also a good prognostic indicator for overall survival (OS) and progression-free survival (PFS) after first-line cytotoxic chemotherapy in the subgroup of patients with EGFR adenocarcinoma who had the wild-type form of the gene [14].

It is essential to categorize lung adenocarcinoma according to its histologic subtype in order to take advantage of recently developed and highly effective treatments for non-small cell carcinoma. These treatments have been shown to be more effective than previous treatments (ACA). TTF-1 is an unreliable marker for ACA in the lung due to its lack of sensitivity as well as its lack of specificity, so it cannot be used to diagnose ACA. Napsin A, which is also referred to as Nap-A, is a functional aspartic proteinase that possesses the potential to serve as an

alternative marker for primary lung ACA. The purpose of this study was to evaluate how well Nap-A and TTF-1 typed primary lung carcinomas in order to type primary lung carcinomas and distinguish primary lung ACA from carcinomas of other sites. In addition, the purpose of this study was to type primary lung carcinomas. According to the results of the study, the Nap-A test was a more sensitive indicator of primary lung ACA than TTF-1 was (87 percent versus 64 percent; P .001). It was found that Nap-A was more specific than TTF-1 for primary lung ACA in comparison to all other tumors, with the exception of kidney tumors (P< .001); this was the case regardless of the type of tumor. According to the findings and conclusions, Nap-A is more effective than TTF-1 in distinguishing primary lung ACA from other carcinomas (with the exception of kidney carcinoma), most notably primary lung small cell carcinoma and primary thyroid carcinoma. With the help of a combination of Nap-A and TTF-1, primary lung ACA (Nap-A(+), TTF-1(+)) can be distinguished from primary lung squamous cell carcinoma (Nap-A(-), TTF-1(-)) and primary lung small cell carcinoma (Nap-A(-), TTF-1(+)) [15].

When performing diagnostic immunohistochemistry, it is customary to analyze CKs 5 and 6 together using bispecific antibodies. This is done routinely. Despite the fact that CKs 5 and 6 are in no way connected to one another in terms of their functionality, this is the case. In order to acquire a deeper comprehension of the diagnostic value of CK5 or CK6 by themselves, tissue microarrays were subjected to immunohistochemistry for the purpose of conducting the study. These tissue microarrays contained over 15,000 different samples of tumors and 608 different samples of normal tissue. The samples of normal tissue came from 76 different types, while the samples of tumors came from 120 different types. The squamous epithelium of normal tissues contained both CK5 and CK6; however, CK5 predominated in the basal layers, whereas CK6 predominated in the suprabasal layers. Both types of CK were present in normal tissue as well. Contrary to CK6, CK5 was the protein that was responsible for staining the basal cells in a variety of different organs. This task was performed in contrast to CK6. Within the tumors themselves, it was discovered that both CK5 and CK6 were present in in excess of 95% of all squamous cell carcinomas. However, other tumor entities showed different results: CK5 predominated in urothelial carcinoma and mesothelioma, whereas CK6 predominated in adenocarcinomas. CK5 was also found to predominate in adenocarcinomas. When CK5 and CK6 were both analyzed together, it was difficult to differentiate between epithelioid mesothelioma and adenocarcinoma of the lung. However, only 12.8% of cases of epithelioid mesothelioma were positive for CK5 by itself, whereas 23.7% of cases of adenocarcinoma were positive for CK5/6. Patients diagnosed with

epithelioid mesothelioma tested positive for both CK5 and CK5/6. Both CK5 and CK6 expressions were found to be associated with high grade, estrogen receptor, and progesterone receptor negativity in breast cancer (all of which had a p-value of less than 0.0001), grade/stage progression in urothelial cancer (all of which had a p-value of less than 0.0001), and RAS mutations in colorectal cancer (all of which had a p-value of less than 0.0001). (all of which had a p-value of less than 0.0001). (each of which had a p-value that was lower than 0.01) Because CK5 is the only factor that is responsible for the useful diagnostic properties that are commonly attributed to CK5/6 antibodies, those properties can only be attributed to CK5, which is the only factor responsible for those properties. One of these properties is the staining of basal cells in the prostate. Other properties include the identification of basal-type features in urothelial cancer, the differentiation of adenocarcinoma of the lung from squamous cell carcinoma and epithelioid mesothelioma, as well as the differentiation of squamous cell carcinoma from epithelioid mesothelioma. It's possible that monospecific CK5 antibodies are a better option than bispecific CK5/6 antibodies when it comes to differentiating between the various types of thoracic tumors. This is something that can be investigated further. [Volkel et al., 16] notes that this is the case, at the very least, if we restrict our attention to the diagnostic procedure.

Study objectives:

The main objectives of the present study were to profile lung cancers from immunohistochemical points of view and to correlate the immune profiling with demographic variables and clinical status of the disease.

METHODS AND SUBJECTS:

Study design and setting:A retrospective study design was conducted to collect data from files of patients using Hakeem electronic system from 2016 to 2022 at Princess Eman Center for Research and Laboratory Science in Royal Medical Services.

Study sample:

A total of 150 files of patients with lung cancer were selected.

Inclusion criteria:

Any file with complete information regarding lung cancer, surgical pathology report, and immunohistochemistry staining was included.

Study variables:

Study variables included gender, age, lung cancer variables, and immunohistochemical profiling.

Statistical analysis:

The analysis of data was carried out using SPSS version 21. Descriptive analysis was used to present data in terms of means and standard deviations, frequencies and percentages for demographic data and clinical data. The relationships between study variables were

examined using Pearson correlation. Significance was considered at alpha level <0.05.

RESULTS:

General characteristics of participants

As shown in table (1), most of patients were males (73.33%) compared with 26.66% females. The age of patients was more than 65 years for 76.33% of patients, whereas the remaining portion of patients was 32.66% for patients less than 65 years. Regarding the smoking status, the results of our data showed that about 73% of patients were smokers compared with nonsmokers (27.33%).

Table 1: General characteristics of participants

Variable	Description
Gender:	
- **Males**	**110 (73.33%)**
- **Females**	**40 (26.66%)**
Age (years):	
- **<65**	**49 (32.66%)**
- **≥65**	**101 (76.33%)**
Smoking:	
- **Yes**	**109 (72.66%)**
- **No**	**41 (27.33%)**

Frequency of lung tumors among study participants

As shown in table (2), adenocarcinoma was the most frequent cancer type (53.33%), followed by squamous cell carcinoma (30%), and neuroendocrine tumors (16.66%).

Table 2: Frequency of lung tumors among study participants

Lung tumor	Frequency (N)	Percentage (%)
Adenocarcinoma	**80**	**53.33%**
Squamous cell carcinoma	**45**	**30%**
Neuroendocrine tumors	**25**	**16.66%**

DISCUSSION

The results of this study showed that males were more likely to have lung cancer. This finding confirms previous studies in which males were more likely to have lung cancer [17]. We think that this may be due to the consideration that males are more likely to be smokers than females.

The age of most patients was more than 65 years. This is in line with other studies that reported age as a risk factor for developing lung cancer [18]. As the age is older, this implies the likely of occurrence of other predisposing factors including the late gene expressions that may participate in lung cancer.

Most patients were smokers. Smoking is an established risk factor for developing lung cancer, particularly in the presence of increasing number of smokers worldwide [19].

The results showed that adenocarcinoma was the most frequent cancer type (53.33%), this finding agrees with other studies in which adenocarcinoma is the most common malignancy in lung tumors [20].

In this study, squamous cell carcinoma (30%) ranked the second. This finding agrees with other studies in which a reduction of the prevalence of squamous cell carcinoma has been observed for unknown reasons. It is plausible that smoking related factors have been improved such as either smoking cessation efforts by patients or industrial improvement through reducing the tar content in smoke [21].

Neuroendocrinetumors (16.66%) came in the last rank. This is in agreement with previous studies [22].

We depended on the expression of biomarkers to profile lung tumors including synaptophysin for neuroendocrine tumors [12, 13], TTF-1 and Napsin A for adenocarcinoma [14, 15], and CK5/6 for squamous cell carcinoma.

CONCLUSION:

Lung cancer patients in this study followed the patterns of western countries. We recommend following smoking cessation policies to reduce the prevalence of lung cancer.

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CONFLICT OF INTEREST REPORTED: NIL; SOURCE OF FUNDING: NONE REPORTED