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Biochemical studies of serum markers in Egyptian patients with non-small cell lung cancer (NSCLC)

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Abstract: Globally, lung cancer is one of the most prevalent and deadly cancers. Exposure to environmental risk factors and genetic susceptibility are also associated with the incidence of the disease. Patients may produce p53 antibodies (p53-Abs) in their serum as a result of p53 gene abnormalities. **Aim**: The study aimed to investigate the utility of detecting serum biomarker such as p53-Abs, carcinoembryonic antigen (CEA), and some other abnormalities characterizing patients with NSCLC. **Methods:** A total of 124 patients with NSCLC and 100 apparently healthy controls were evaluated for measuring serum p53-Abs and CEA levels by ELISA. **Results**: NSCLC patients presented with specific characteristics**.** The histological assessments were classified as (79.9%) LUAD, (8.9%) LUSC, LCC (6.4%) and (4.8%) other histological cell types with histological grades divided as grade I (1.7%), grade II (34.2%) and grade III (64.2%). The immuohistochemical analysis revealed that (51.6%) of patients were positive for TTF1, (70.2%) positive for CK7 and (33.6%) positive for napsin. Patients were also had significant elevated levels of p53-Ab, and CEA, compared to the controls ($p<0.05$). **Conclusions**: Detection of serum p53-Abs and CEA levels are important tumor markers characterizing NSCLC. P53 can be served as possible biomarker for the occurrence of p53 gene mutation in NSCLC patients.

keywords: Lung cancer, NSCLC, biomarkers, p53-Abs, CEA.

1.Introduction

With around 2.1 million new cases annually (or 12% of all newly diagnosed cancers), lung cancer is the most prevalent disease diagnosed worldwide. It is also the leading cause of death from cancer, accounting for about 1.8 million fatalities annually (1).

Global Cancer Data (GLOBOCAN) observation in December 2020 reported that the largest number of cases of specific cancers in Egypt was liver, followed by breast cancer. The prevalence of lung cancer comes fifth after bladder and non-Hodgkin lymphoma, with a total of 134,632 for all malignancies. The incidence of lung cancer in males is approximately 2:1 among females. The highest death numbers for 2020 in Egypt were liver, breast, bladder, lung, and non-Hodgkin lymphoma, with a total of 89,042 for all cancers (2).

The two chief types of lung cancer are: non-small cell lung cancer (NSCLC) accounts for more than 80–85% of lung cancers and small cell lung carcinoma (SCLC) represents 12% of all lung cancer cases. SCLC is characterized as a neuroendocrine cancer because the cancer cells contain features of neurons and endocrine (hormone-secreting) cells and are less diffuse but more lethal and heterogeneous than NSCLC (3).

Although smoking is a major risk factor for developing lung cancer, non-smokers also suffer from this disease. Other factors have been associated with an increased risk of lung cancer development, as well as a family history of pulmonary tumors, age, gender, race, environment, occupational exposure, lifestyle habits, and genetic and epigenetic factors (4).

One important regulator of development involved in normal cell cycle control pathways including growth arrest and apoptosis is the tumor suppressor gene p53. Tumor cells accumulate mutant p53 proteins because they have a significantly higher half-life than the wild-type protein. The accumulated proteins may be liberated from the tumor cells, whereupon they will be recognized as foreign proteins by the human immune system, triggering the humoral reaction that will result in the formation of antibodies against the proteins. The existence of p53-Abs and p53 accumulation or mutation in the tumor is often highly correlated. Accordingly, the identification of p53-Abs may function as potential indicators of p53 gene mutation. (5).

There is currently a strong link between the particular development of anti-p53 antibody titers and some types of cancers, according to a number of studies that have sought to assess the clinical use of anti-p53 antibodies including colorectal cancer (6), breast cancer (7) and ovarian cancer (8). This study aimed to investigate the main serum biomarker and histopathological abnormalities characterizing patients NSCLC in Egypt.

2. Materials and methods

Study population

The current investigation, a case-control study, was carried out on 124 patients at the Oncology Center of Mansoura University (OCMU) with non-small cell lung cancer (NSCLC). It was approved by the Mansoura University Faculty of Medicine Ethics Committee (MS. 23.11.2629) and all participants provided written informed permission. As a control group, hundreds of unrelated, healthy, cancer-free volunteers of the same age and sex were enlisted. This study was conducted between October 2023 and June 2024. Relevant clinical data were retrieved from registered data archives. Demographic data such as age at cancer onset, family history of cancer, smoking, and parental relatives were also taken from the patients using a specific form. The inclusion criteria involved age >20 years (at the time of NSCLC diagnosis for cases and at the time of sampling for controls). Participants with autoimmune disorders and viral infections were excluded from this study. No one in the control group was a smoker, had a history of any emerging disease, chronic use of any medications, or features or family history suggestive of NSCLC.

Methods

For both the patient and control groups, five milliliters of peripheral blood were aspirated from each participant. Every blood sample was split into two aliquots: the first, containing two milliliters, was put into sterile EDTA tubes for the hematological analysis, and the second, containing three milliliters, was taken in plain tubes for the biochemical analyses.

Immunohistochemistry (IHC) analysis

Pathological findings related to immunohistochemical staining Transcription termination factor-1 (TTF-1), cytokeratin 7 (CK7), and Napsin were extracted from the electronic medical archives

Enzyme-linked immunosorbent assay analysis (ELISA)

Serum levels of p53 antibodies were measured using a human p53 quantitative ELISA assay (BMS256-BenderMedSystems, Austria), while serum CEA level was measured using human ELISA Kit (catalog no. EHCEA. 0244081221A; Invitrogen, Thermo Fisher Scientific), according to the manufacturer's protocol.

2.2.3. Biochemical and hematological measurements

Biochemical measurements, including serum ALT, AST, total bilirubin, albumin and creatinine were assessed using endpoint/kinetic assay kits according to the manufacturer’s instructions. A complete blood count was analyzed using an automated analyzer.

Statistical analysis

The acquired data was categorized, and tabulated using the SPSS (IBM Corporation, IBM SPSS, 2017). Parametric numerical data was expressed by mean (± SD), non-parametric data was expressed by median (min-max) and the non-numerical data was expressed by percentage and frequency. Student-t test was used to evaluate the statistical significance of the difference between study group parametric variables. The Mann-Whitney U test was used to determine the statistical significance of a

non-parametric variable of study groups. Chi-Square and Monte Carlo tests were used to examine the relationship between qualitative variables as appropriate. P-value is stated significant if < 0.05.

3. Results and Discussion

Results

Totally, 124 NSCLC patients were investigated with mean age 56 ± 11.5y (59.7% males and 40.3% females). The control group contained 100 healthy individuals with mean age 55.4 ± 11.0 y (64% males and 36% females). No statistical significant difference was found between the two groups regarding age and sex (p=0.5 and 0.66, respectively). In NSCLC group, 11.3% of the patients had a family history to the disease. Regarding the smoking status, 49.2% of the patients were non-smokers, while 9.7% were passive smokers and 41.1% were active smokers.

NSCLC patients were presented with different symptoms including cough, dyspnea, chest pain, blood sputum, fever, bronchitis, and hemiplegia (**Figure 1).** The results of the histopathological assessments of the patients were presented in (**Table 1).** NSCLC patients were classified as (79.9%) LUAD, (8.9 %) LUSC, LCC (6.4%) and (4.8%) other histological cell types with histological grades divides as grade I (1.7%), grade II (34.2%) and grade III (64.2%).

Results of chest examination were displayed in (**Table 2)**. Fifty-four percent of patients experienced pleural effusion, and 50.8% lung collapse. Using IHC markers we observed that (51.6%) of patients were positive for TTF1, (70.2%) positive for CK7 and (33.6%) positive for napsin, as shown in (**Table 3).**

Moreover in (**Table 4)**, regarding the lab measurements, the WBCs, relative neutrophils, neutrophil-lymphocyte ratio (NLR), platelets count, AST activity, Hb concentration, creatinine, CEA and p53-Abs levels were significantly higher in NSCLC patients compared to control (p<0.001 for all except p=0.022 for AST and p=0.02 for creatinine), while the albumin level and relative lymphocytes count were significantly decreased in the NSCLC group compared to the controls (p<0.001 for both).

Discussion:

According to World Cancer Statistics 2020, lung cancer cases reached 2.2 million cases among the entire population, compared to 6,538 cases among Egyptians (9).

Recently, a number of studies have clarified particular biomarkers linked to cancer diseases or abnormalities. According to these studies, lung cancer is a complicated disease that develops from the aberrant expression or mutation of numerous essential genes. As a result, different genes should be assessed differently globally and maybe based on statistically significant findings (10). The tumor suppressor gene P53 is one of the most commonly altered genes in cancer (11).

In this case-control study, the mean age of patients at the time of hospitalization and diagnosis of lung cancer was 56.0 ± 11.5 y. Regarding gender, males accounted for 59.7% of patients, while females accounted for 40.3%. This observation coincided with that study presented by **Ghoneim et al.,** who found that the mean age of the studied cases was 57.08 ± 11.4 y with a higher male predominance of 75.7% (12). The fact that men are more likely to be exposed to smoking and pollution as well as occupational hazards explains the relative male predominance in lung cancer risk (13).

Smoking is the highest risk factor of lung cancer. We found that, 41.1% of the patients were smokers, 9.7% were passive smokers, and 49.2% were never smokers; however, current smokers accounted for less than half of NSCLC cases. This finding was inconsistent with a previous study, which found that smoking was responsible for more than 60% of lung cancer cases (14). This disparity may be explained by the fact that the cases were of different genders.

Lung cancer occurs as a result of local tumor growth, regional spread via the lymphatic system, distant hematogenous metastases, and distant paraneoplastic effects from tumor products or cross-reactivity. In this study, and similar to previous a study by Alamoudi who found that cough represented 39.5% of patients, was the most prominent symptom. Cough may be caused by local growth in a central location, or it may be a feature of large airway obstruction causing post-obstructive pneumonia or lymphadenopathy (15).

Dyspnea develops early in lung cancer. It may be caused by large airway obstruction, pleural effusion, lymphatic embolism, phrenic nerve palsy, severe lung disease, or cardiac and pericardial involvement (16)**.** In this study, dyspnea was present in up to 33.9% of patients, a result lower than previously reported rates of 60% by Al-Jahdali. This disparity may be attributed to climatic differences in the study areas, which contribute significantly to respiratory problems (16).

Chest pain at the time of diagnosis is often the result of pleural infiltration, rib metastases, or direct invasion of the ribs or vertebrae by tumors. In the presenting NSCLC patients, chest pain complaints were consistent with those occurring in only 32.3% of patients in the study by Al-Jahdali (16). Hemoptysis as streaks of blood in the sputum was seen in 6.5% of patients, which is lower than previously reported by Al-Amoudi, which represented one-third of patients with lung cancer. This may be attributed to smoking-related bronchitis (15). Moreover, according to a study conducted at Aswan Hospital, the most prominent symptom was shortness of breath, cough and spitting up blood, while wheezing and chest pain were the least common symptoms (17).

Chest radiography is the most common method of detecting lung cancers; unfortunately, most of these instances are advanced when they are first discovered. (18)**.** In the current study, the right lung was more frequently affected than the left (61% vs. 50%, respectively). Notably, this finding is consistent with the study conducted by Al-Amoudi (15). However, there is no scientific explanation for the preference for the right lobes of the lung. Lung tumors may also cause fluid accumulation in the lung or the space surrounding the lung, or force air out of the lungs and cause lung collapse. We observed from the chest examination results that 54% of patients experienced pleural effusion, and 50.8% lung collapse.

In fact, the presence of vascular invasion by malignant cells means that the malignant tumors have spread. Blood vessel invasion has been associated with disease progression in a number of different malignancies (19). Of all 124 NSCLC patients, 19.5% of the cases showed vascular invasion. In a meta-analysis that included 16,535 participants from 52 relevant trials, the blood vessel invasion was seen in 29.8% of patients with a mean incidence of 6.2% to 77.0% (20)**.**

The diversity of symptoms as well as clinical signs and their incidence are usually determined by the representation of histopathologic subtype, tumor location, and stage of lung cancer at diagnosis. The three major histologic subtypes of NSCLC include LUSD (25%–30%), LUAD (40%), and LCC (10%) of all lung cancers (21)**.** In this study, LUAD was the prominent subtype (79.0%).

Our findings revealed no significant difference between the NSCLC and control group with respect to some lab measurements. The platelets count, AST activity, Hb concentration, creatinine and CEA levels were significantly higher in NSCLC patients compared to control ($p<0.05$).

According to previous studies, serum tumor markers can indeed be suspected in cancer cases, in addition to the presence of cancer at an unclear primary site. Serum tumor markers, including CEA, are clinically used to screen and assess recurrence of NSCLC and linked to higher TNM stage. The normal range of CEA is 0 to 2.5 ng/ml. Anything >10 ng/ml indicates extensive disease, and levels >20 ng/ml indicate that the cancer may have spread (22). However, some previous studies reported that CEA is non-specific for detecting lung cancer with only 40–70% sensitivity for detecting NSCLC, which leads to poor clinical diagnostic efficiency in the early disease stage (23). Low sensitivity was observed in investigating the CEA level of NSCLC patients in the present study, with the median CEA level ranging from 1-1500 ng/ml.

We also observed that WBCs, relative neutrophils and neutrophil-lymphocyte ratio (NLR) were significantly higher in NSCLC patients in comparison with the controls ($p<0.05$), while the albumin level and relative lymphocytes count were significantly decreased in the NSCLC group compared to the controls ($p<0.05$). Inflammation is recognized as a condition that contributes to cancer formation and a condition that arises as a result of carcinogenic changes in cancer cells**.** There are

many laboratory studies that have been conducted on inflammation. Markers implicating systemic inflammation, such as plasma CRP concentration, low albumin levels, total white blood cell count and neutrophils or NLR (24).

Interestingly, researchers have demonstrated that the NLR has independent prognostic significance in a number of malignancies, such as lung cancer, colorectal cancer, pancreatic cancer, and ovarian cancer (25-27). These investigations found that the prognostic significance of the NLR was independent of tumor stage, treatment modalities, and traditional classification systems. High median NLR was significantly elevated in NSCLC group, providing evidence to be an independent unfavorable prognostic factor in such patients ($P < 0.001$). This result was consistent with Zhang et al., who investigated the clinical significance of neutrophil-to-lymphocyte ratio in primary NSCLC patients (28).

Moreover, our results revealed that p53-Abs levels were significantly higher in NSCLC patients compared to control ($p<0.001$). Prior research has demonstrated that in patients with lung cancer, tumor antigens trigger a particular type of immune response. The most researched tumor antigen in this category is p53-Abs. Tumor suppressor gene p53 mutations can trigger an immune response, and p53-Abs can appear early in some cancers' carcinogenic processes (29).

P53-Abs elevatation was in alignment with the study by Hastalıklar et al., which indicated that the presence of p53-Abs in the serum of patients with NSCLC is useful in identifying those with poor prognosis (30).

Fontanini et al. determined that serum mutant p53 levels in 13% of patients, and p53 concentrations were significantly rised in patients with lymph node involvement and advanced stage (31).

Our findings were also in agreement with the study performed by **Shaolei et al.,** who informed that the rate of p53-Abs in patients with various malignancies was higher than the controls. They proposed using serum p53-Abs in the early diagnosis of malignancies (32).

In conclusion**,** Detection of serum p53-Abs and CEA levels are important tumor markers characterizing NSCLC. P53 can be served as possible biomarker for the occurrence of p53 gene mutation in NSCLC patients.

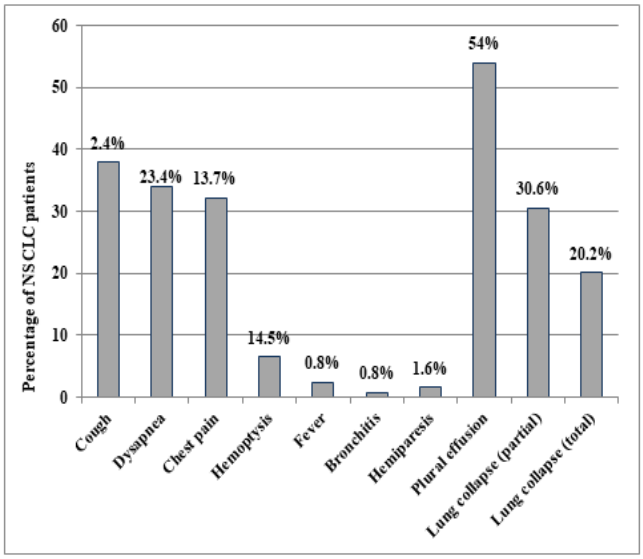

Figure 1: Clinical presentation of NSCLC cases.

Table 1: Pathological assessment of the NSCLC patients.

Parameters	n (%)
Tumor location	
-Right side	48 (38.7%)
-Left side	45 (36.3%)
-Bilateral	31 (25%)
Tumor histopathology	
-LUAD	99 (79.9%)
-LUSD	11 (8.9%)
-LCC	8 (6.4%)
-NOS	6 (4.8%)
Histological grade	
-Grade I	2 (1.7%)
- Grade II	42 (34.2%)
- Grade III	80 (64.2%)
T stage	
-T1	2 (1.6%)
-T2	9 (7.2%)
-T3	58 (46.8%)
T4	55 (44.4%)
N stage	
-N0	23 (18.5%)
-N1	7 (5.6%)
-N2	56 (45.1%)
-N3	38 (30.8%)
M stage	
-M0	30 (24.2%)
-M1	94 (75.8%)

LUAD: Lung adenocarcinoma, LUSC: Lung squamous cell carcinoma, LCC: Large cell carcinoma.

Table 2: Chest examination of NSCLC cases.

Characteristics		NSCLC (n=124)	
		n	%
Pleural effusion	Yes	67.0	54%
	No	57.0	46%
Lung collapse	No	61.0	49.2%
	Partial	38.0	30.6%
	Total	25.0	20.2%

Parameters were expressed as frequency (percentage).

Table 3: Immunohistochemistry results of NSCLC group.

IHC		NSCLC (n=124)	
		n	%
TTF1	**No**	60.0	48.4%
	Yes	64.0	51.6%
CK7	**No**	37.0	29.8%
	Yes	87.0	70.2%
Napsin	**No**	82.0	66.1%
	Yes	42.0	33.6%

TTF1: Thyroid Transcription Factor-1, CK7: Cytokeratin 7.

Table 4: Comparison of lab measurements between NSCLC patients and controls

Parameters	NSCLC (n = 124)	Control (n=100)	p-value
Biochemical measurements			
ALT (U/L), Median (Range)	21 (4 – 127)	21 (11 – 35)	0.76
AST (U/L), Median (Range)	21 (9 – 130)	19 (10 – 33)	0.022*
Albumin (g/dl), Mean ± SD	3.63 ± 0.53	4.49 ± 0.46	<0.001*
Creatinine (mg/dl), Mean ± SD	0.94 ± 0.77	0.75 ± 0.23	0.02*
CEA (ng/ml), Median (Range)	6.0 (1 – 1500)	2.7 (2.2–3.4)	<0.001*
P53 (pg/ml), Median (Range)	284.8 (182.3–297)	78.2(65.1–92)	<0.001*
Hematological measurements			
RBCs ($\times 10^{12}$/L), Mean ± SD	4.74 ± 0.66	4.63 ± 0.66	0.21
Hemoglobin (g/dl), Mean ± SD	12.8 (7.06 – 17.3)	12.0 (10.0 – 15.0)	<0.001*
WBCs ($\times 10^{9}$/L), Median (Range)	9.88 (3.8 – 36.4)	6.0 (3.8 – 10.0)	<0.001*
Lymphocytes(%), median (range)	22.0 (3.97 – 57.6)	25.95 (15.9 – 45.9)	<0.001*
Neutrophils (%), median (range)	65.9 (38.9 – 89.77)	56.0 (25.78 – 62.3)	<0.001*
NLR, median (range)	3.01 (0.67 – 22.29)	2.19 (0.89 – 3.29)	<0.001*
Platelets ($\times 10^{9}$/L), Median (Range)	295.5 (37.64 – 542.1)	252.0 (160 – 380)	<0.001*

Student t-test, Mann-Whitney U-test and Chi-square test were applied; *: Significant at p<0.05, WBCs: White blood cells, RBCs: Red blood cells, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, NLR: Neutrophils/lymphocytes ratio, , CEA: Carcinoembryonic antigen, TTF1: Thyroid Transcription Factor-1, CK7: Cytokeratin 7.

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