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Role of annexin A1 in early diagnosis of lung cancer

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Abstract---Background: The prevalence of lung cancer has shown an increase over the last few years which cause both health and economic burden. The biopsy is the gold standard tool for disease diagnosis, but it is usually not accepted by the patients due to its invasive nature. The use of non-invasive biomarkers is now attaining a great interest in diagnosis. Aim of the work: Assess the role of Annexin A1 in bronchoalveolar lavage and serumin the early diagnosis of lung cancer. Patients and methods: This study included 39 patients into two groups; group A (cases with lung cancer) and group B (cases with non-malignant lung lesions). All subjects were submitted to history taking and thorough full physical examination and laboratory analysis. Bronchoalveolar lavage (BAL) was performed in the cases within the two groups. Both serum and BAL levels of annexin A1 were assed in all cases. Results: In the current study, the level of annexin A1 in the serum and BAL were statistically significantly higher in the malignant group as compared with the non-malignant group (P□ 0.001). The diagnostic parameters were higher in annexin A1 in the

BAL as compared to the serum annexin A1 in differentiating lung cancer from suspicious non-malignant lung lesions.In the current study, the best cutoff point of Annexin A1 in BAL in identifying the cases with malignant lesions was > 10.75 with 85% sensitivity and 57.9% specificity. The best cutoff point of Annexin A1 in serum in identifying the cases with malignant lesions was 9.464 with 85% sensitivity and 57.9% specificity. Conclusion: Annexin A1 could be a sensitive marker in the diagnosis of lung cancer especially the BAL annexin A1 which revealed higher diagnostic accuracy compared to the serum annexin A1.

Keywords---lung cancer, BAL, annexin A1.

Introduction

According to the 2020 World Cancer Report, lung cancer continues to cause the grand majority of cancer- related mortality (18%) [1] . Approximately two million new cases are diagnosed each year worldwide [2] . Lung cancer is histologically classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC represents around 85% of all lung cancers and comprises two predominant histological subtypes: adenocarcinoma (AC, 65%) and squamous cell carcinoma (SQCLC, 30%) [3] Bronchoscopy has been widely applied in the diagnosis and treatment of lung cancer. At present it is considered the main and most effectivemethod for diagnosing patients with a suspicion of a malignancy in the lung. The diagnosis often depends on a tumor tissue biopsy; however, sometimes it is difficult to obtain lung cancer tissues for pathological diagnosis [4, 5] . The bronchoscopy operator may sample bronchoalveolar lavage fluid (BALF) for further testing to help in diagnosis. BALF has been regarded as an alternative resource for detecting biomarkers of lung cancer because of its vicinity to tumor tissues and cells [6]

A number of studies using BALF for detection of markers represented a source of lung cancer-specific protein biomarkers, which was noninvasive with elevated diagnostic reliability and still needed to be increased [7] . Annexin A1 (Anxa1) is the first agent of the superfamily of Annexinproteins which are phospholipid- and calcium-binding proteins [8]. Anxa1 is an intracellular mediator of glucocorticoids´ anti-inflammatory action via phospholipase A2 inhibition [9]. Deregulation of Anxa1 levels and alterations to its subcellular localization have been linked to the development or even the progressionof various types of cancers. High levelsof Anxa1 expression have been found in bronchogenic carcinoma [10] , hepatoma [11] , pancreatic cancer [12] , colorectal cancer [13] and in melanomas [14] . However, the study of the expression level of annexin A1 in BALF and its significance in the development of lung cancer is considerably limited.

Patients and Methods

This is a cross sectional case control study that was conducted at Chest and the Medical Biochemistry and Molecular Biology Departments Faculty of Medicine Menoufia University Hospitals, from March 2021 to April 2022. This study

included a total of 39 subjects who into two groups; Group A (Malignant group) (that included twenty patients diagnosed as lung cancer by histopathological examination via different diagnostic methods based on WHO by FOB, U/S, CT guided biopsy) and group 2 (the non-malignant group) (that includes nineteen patients having non-malignant lung diseases, were diagnosed as pulmonary tuberculosis, bronchiectasis, lung abscess and pneumonias).

We included the patients from both genders aged more than 18 years with lung cancer or lesions suspicious to lung cancer who were fit for fiberoptic bronchoscopy (FOB) or transthoracic lung biopsies. The study excluded the cases with the following criteria; uncooperative or unfit patients to FOB or transthoracic lung biopsy, patients with severe pulmonary hypertension or uncorrectable coagulopathy, patients with insufficient biopsies, other co- existing malignancies as hepatic and pancreatic cancers, presence of pulmonary metastases, patients who received chemotherapy and/or radiotherapy, recent myocardialinfarction and patients who refused to participate. The study is conducted in accordance with Helsinki Standards as revised in 2013 [15] . The study was conducted after obtaining the approval from the local ethics committee, Faculty of Medicine, Menoufia University and afterobtaining a written/oral informed consent from the included cases.

The cases were subjected to the following; history taking and thorough full physical examination with emphasizing on the local chest examination. Laboratory investigations were also performed in the cases. Radiological assessment included plain chest X-ray postero-anterior and lateral views and CT Scan of the chest (incertain cases). Serum and BAL were measured by annexin A1 (ANXA1) ELISA detection kit, [Sunred biomedical company, Shanghai, China (Assay range : 0.3ng/ml →90ng/ml).

Serum preparation

Five milliliters venous blood sample was withdrawn from each patient in this study by sterile venipuncture, and were delivered into plain tube and left for clotting then, after centrifugation at 3000 rpm for 15 minutes for the serum preparation, thenwere numbered and stored at −80°C.

Bronchoalveolar lavage:BAL collection

Bronchoscopy was done using PENTAX bronchoscope EB012780 (PENTAX Medical, Tokyo, Japan), with a 3.2-mm working-channel diameter and 60-cm working length equipped with biopsy forceps. Radiographs were reviewed to detect the best site of alveolar lavage. Bronchoscope and collection trap were prepared. Patients at risk of bronchospasm were premedicated with bronchodilators. The patient was best positioned in supine position when oncoming lingual or right middle lobe. Supplemental oxygen was applied. Lidocaine 2% was used. Intravenous midazolam (0.01–0.1 mg/kg) was the sedative drug that can provide the required sedation. Bronchoscope was progressed until wedged in the wanted site. Twenty milliliters of saline was poured with a syringe. Suction was avoided, until irrigation distillation was completed and gentle suction (50–80 mmHg) was applied. Then, the fluid specimens were collected in thecollection trap. Steps 3, 4,

and 5 were repeated, up to five times to obtain an adequate specimen (40–60 ml). Thepatient was observed and monitored for about 1 h after the procedure [16].

Preparation of bronchoalveolar lavage fluid sample [17]

BALF samples were collected in a siliconized container. The fluid was filtered and centrifugation was done at 4°C, 3000 rpm for 30 min to separate the floating impurities. The samples were numbered and stored at −80°C.

Statistical analysis

The data collected were coded, processed and analyzed with SPSS version 26 for Windows® (Statistical Package for Social Sciences) (IBM, SPSS Inc, Chicago, IL, USA). Qualitative data as number (frequency) and percent was presented. The Chi-Square test (or Fisher's exact test) made the comparison between groups. The Kolmogorov-Smirnov test tested quantitative data for normality. Data was shown as median ± SD. To compare two groups with categorical variables, Chi-Square test (or Fisher's exact test) were used. To compare two groups with normally distributed quantitative variables, independent samples (student's) t-test was used and Mann-Whitney U-test was used if the data were abnormally distributed. The optimal cutoff value of annexin A1 to differentiate between different groups was determined using Youden index J that is the farthest point on receiver operator characteristic (ROC) curve and expressed in terms of sensitivity and specificity. For all tests, P values <0.05 are considered significant.

Results

The current study included 20 cases with lung cancer lesions (Malignant group) in addition to 19 cases with non-malignant lung diseases. Table (1) shows that there was no statistically difference between the malignancy and the non-malignant group regarding the age and gender. The highest percentage of the malignant group were males (75%). The prevalence of smoking was statistically significantly higher in the malignant group (70% vs 36.8%) than in the non-malignant group. There was no statistically significant difference between the two groups regarding the prevalence of DM and HTN. There was no statistically significant difference between the two groups regarding the presenting symptoms with cough as the most common presenting symptom.

Table 1
Analysis of the demographic data, risk factors, comorbidities and clinical presentation in the two study groups

	Groups				P value
	Malignant group (n = 20)		Non-malignant group (n = 19)		
Age (Years)	53.85 ± 11.58		46.89 ± 16.39		0.133
Gender					
Male	15	75 %	9	47.4 %	0.076
Female	5	25 %	10	52.6 %	

Smoking	14	70 %	7	36.8 %	0.038*
DM	4	20 %	3	15.8 %	0.723
HTN	2	10 %	3	15.8 %	0.589
Cough	18	90 %	15	78.9 %	0.339
Dyspnea	9	45 %	11	57.9 %	0.421
Haemoptysis	6	30 %	5	26.3 %	0.798
Chest pain	9	45 %	8	42.1 %	0.855
*: Statistically significant (p 0.05)					

Table (2) shows that the presence of mass was statistically significantly higher in the malignant group (90% vs 10.5%) than in the non-malignant group (p< 0.001). The presence of cavity was statistically significantly higher in the non-malignant group (47.4% vs 10%) than in the malignant group (p= 0.010). The presence of positive LNs was statistically significantly higher in the malignant group (40% vs 5.3%) than in the non-malignant group (p= 0.010). The presence of Endobronchial odema was statistically significantly higher in the malignant group (70% vs 0%) than in the non- malignant group (p< 0.001). The presence of edema was statistically significantly higher in the malignant group (70% vs 10.5%) than in the non-malignant group (p< 0.001). There was no statistically significant difference between the prevalence of consolidation in the two groups.

Table 2
Analysis of clinical and radiological findings in the two study groups

	Groups				P value
	Malignant group(n = 20)		Non-malignantgroup (n = 19)		
Mass	18	90 %	2	10.5 %	< 0.001*
Cavity	2	10 %	9	47.4 %	0.010*
Consolidation	10	50 %	12	63.2 %	0.408
LNs	8	40 %	1	5.3 %	0.010*
Endobronchial odema	14	70 %	0	0 %	< 0.001*
Edema	14	70 %	2	10.5 %	< 0.001*
*: Statistically significant (p 0.05)					

Table (3) shows that the annexin A1 level in BAL and annexin A1 in serum were higher in the malignant group as compared to the control group, with high statistically significant difference between the two groups.

Table 3
Analysis of annexin A1 in BAL and serum in the two study groups

	Groups		P value
	Malignant group(n = 20)	Non-malignant group (n = 19)	
Annexin A1 inBAL	16.045 (8.12- 56.397)	9.92 (5-11.479)	< 0.001*
Annexin A1 in serum	18.683 (8.923- 90)	8.905 (4.984-14.391)	< 0.001*
*: Statistically significant (p 0.05)			

Table (4) shows that, in the malignant group, Bronchoscopic biopsy was the method for diagnosis in 65% of the cases, CT guided biopsy in 15%, ultrasound guided biopsy in 10% while cytology by BAL (10%). In the non-malignant group, BAL culture and sensitivity was the method for diagnosis in 52.6% of the cases, Ziehl–Neelsen (ZN) stain in 42.1% and sputum culture in one case (5%). This table shows that small cell carcinoma was the most common pathological diagnosis in the malignant group in 40% of the cases followed squamous cell carcinoma in 35% and at last adenocarcinoma in 25%. This table shows that TB was the most common lesion in the non-malignant group in 42.1% of the cases followed by pneumonia in 31.6%, lung abscess in 21.1% and at last COPD in one case only (5.3%).

Table 4
Method of diagnosis and pathological results in the two study groups

Mode of diagnosis		
Malignant group (N=20)		
	Number	Percent
Bronchoscopic biopsy	13	65
CT guided biopsy	3	15
Ultrasound guided biopsy	2	10
Cytology of BAL	2	10
Non-malignant group (N=19)		
BAL culture and sensitivity	10	52.6
Ziehl–Neelsen (ZN) stain	8	42.1
Sputum culture and sensitivity	1	5.3
Pathological findings		
Malignant group (N=20)		
Small cell carcinoma	8	40
Squamous cell carcinoma	7	35
Adenocarcinoma	5	25
Non-malignant group (N=19)		
TB	8	42.1
Pneumonia	6	31.6
Lung abscess	4	21.1
COPD	1	5.3

The best cutoff point of Annexin A1 in BAL in identifying the cases with malignant lesions was > 10.15 with 85% sensitivity and 57.9% specificity (Table 5, Figure 1). The best cutoff point of Annexin A1 in serum in identifying the cases with malignant lesions was 9.464 with 85% sensitivity and 57.9% specificity (Table 5, Figure 2).

Table 5
Predictive value of annexin A1 in BAL and serum in identifying malignantlesions

Diagnostic criteria	Annexin A1 in BAL	Annexin A1 in serum
AUC	0.895	0.824

Cut off point	> 10.75	> 9.464
P	0.001*	0.001*
Sensitivity	85 %	85%
Specificity	84.2 %	57.9%
PPV	88.6 %	52.4%
NPV	80.4 %	82.3%
Accuracy	86.2 %	78.2%
*: Statistically significant (p 0.05)		

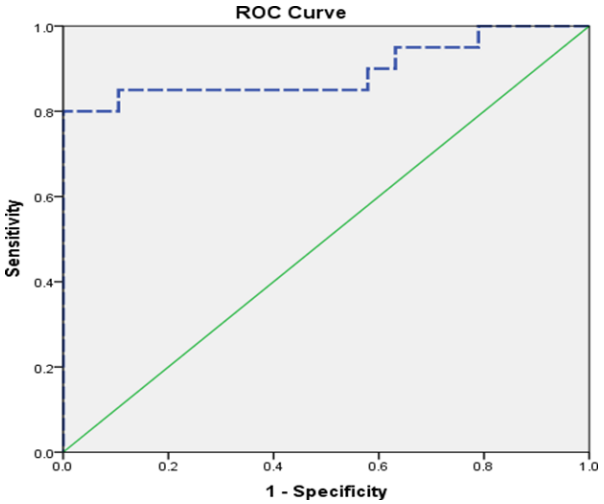

Figure 1. ROC curves of annexin A1 in BAL in identifying cases with malignant lesions

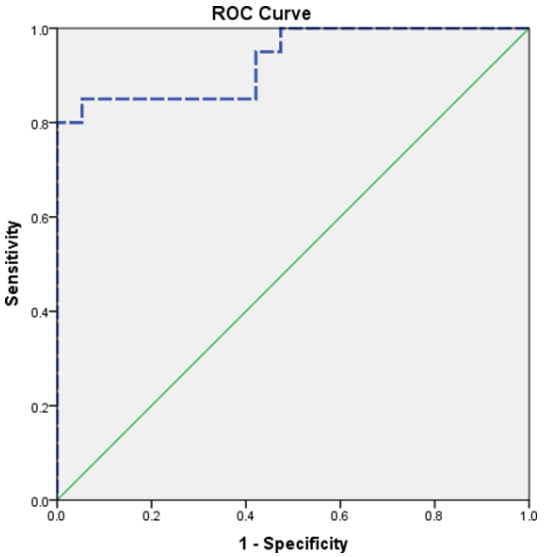

Figure 2. ROC curves of annexin A1 in serum in identifying cases with malignant lesions

Discussion

The current study included 20 cases with lung cancer lesions (Malignant group) in addition to 19 cases with non-malignant lung diseases. The current results revealed that there was no statistically difference between the malignancy and the non-malignant group regarding the age and gender. This eliminates the selection bias regarding the age or the gender. However, the current study revealed a male predominance where males represent 75% of the included cases with lung cancer. This agreed with Kumar et al. who include 125 patients with primary lung cancer. The authors showed that out of 125 cases of primary lung cancer studied, 81 (64.8%) were males and 44 (35.2%) were females. The male to female ratio was 1.84:1 [18] . Also, in accordance with the current results, an Egyptian study conducted by Sayed and his colleagues in Aswan showed that males were 76.7% and females were 23.3% from the cases with lung cancer included [19]

The relative male predominance in lung cancer risk can be explained by the fact that males are at higher risk of exposure to smoking, air pollution and occupational hazards. It is now assumed that there is sex difference in their susceptibility to carcinogenic effects of tobacco smoke. This variation may be due to difference in DNA repair mechanisms [20] . In the current study, the mean age in the cancer group was 53.85 ± 11.58 years. Mean age of our patients is comparable to the studies done on clinical profile in lung cancer patients in other countries [19, 21-23] . Smoking is always attributed to lung cancer development [24, 25] . In an Egyptian study, almost two/third of the patients were smokers; most of which were cigarette smokers. Smoking was prevalent mainly in the urban population [26]. This was confirmed in the current study, where the prevalence of smoking was statistically significantly higher in the malignant group (70% vs 36.8%) in the non-malignant group.

In the current study, there was no statistically significant difference in the presenting symptoms between the cases with malignant and non- malignant lung lesions. The most common presentation of lung cancer were cough (90%), dyspnea and chest pain (45% for each) and haemoptysis in 30% of the cases.In agreement with the current results, Kumar and his colleagues reported that cough was the most common presenting symptom and was present in 103 out of 125 (82.4%) patients who were included in the study, followed by dyspnea (76.8%), anorexia and weight loss (60.8%), and chest pain (42.8%) [18]. Comparable to our results, Buccheri &Ferrigno stated that their patients at presentation, experienced two or three symptoms on average; the most predominant being cough and systemic symptoms followed by dyspnoea, chest pain and haemoptysis, similarly [27] . Beckles et al. stated that cough was the most common presenting symptom of lung cancer followed by dyspnea and hemoptysis [28] . Moreover, Patil &Rujuta summarized that the most commonly experienced symptoms in their study on 210 patients were cough, hemoptysis and dyspnea [29] .

In the current study, mass, endobronchial odema, consolidation were statistically significantly higher in the malignant group. These symptoms represent 90%, 70% and 50% of the cases within the malignant group respectively. Sayed et al. reported that more than 80% of patients with lung cancer in their study

presented with mass and 40% with collapse, less common radiological presentation include consolidation, effusion, nodules and other radiological findings in the form of 1 patient with mediastinal lymph nodes and the other one with malignant lung abscess Moreover, some patients have more than one of the previously mentioned radiological presentations [19]. A recent study by Gebremariam et al. reported that the most prominent radiologic reported features in cases with lung cancer were lung mass (84.9%, n = 124), pleural effusion (52.7%, n = 77), multiple lung nodules (43.8%, n = 61), extrathoracic metastasis (41.8%, n = 61), atelectasis or consolidation (32.2%, n = 47), and lymphadenopathy (18.5%, n = 27) [30] .

In agreement with our results Rawat et al. stated that mass lesion was the commonest radiological presentation followed by collapse- consolidation, then pleural effusion and combined presentation [31] . Also, Patil &Rujuta reported that radiological patterns of abnormalities documented in their study were mass lesion (29.04%), hilar opacity (27.14%), collapse (segmental/lobar, 20.95%), and pleural effusion (12.38%) [29]. In current study, (65%) of cases diagnosed by bronchoscopic biopsies, and this agreed with (V H Mak., etal., 1990) who make study on Value of washings and brushings at fibro-optic bronchoscopy in the diagnosis of lung cancer. and their result biopsy specimen gave positive result in 76% of cases who have lung cancer.

The variation in radiological presentation between different studies is caused by the variability in tumour stages at the time of diagnosis and the histopathological diagnosis. In the current study, the annexin A1 level in BAL and annexin A1 in serum were higher in the malignant group as compared to the control group, with high statistically significant difference between the two groups. Our results came in accordance with Shepl et al. (2021) who conducted a prospective analytic controlled case study was performed in Chest Department, Tanta University Hospitals, on 40 patients. The patients were split into two groups: group 1 included 20 patients with bronchogenic carcinoma; group 2 included 20 patients having nonmalignant lung diseases of known etiologies. The results showed that annexin A1 levels were higher in BALF and sera of lung carcinoma patients than those of the control group. Positive correlation was present between annexin A1 level in BALF and serum and advanced clinical stages of lung cancer and between BALF annexin A1 level and its level in serum [32].

Moreover, according to Biaoxue et al., annexin A1 levels were measured by ELISA in BALF and serum specimens of 86 lung cancer patients and 41 patients with benign lung disease. The results showed that BALF and serum annexin A1 levels were higher in lung cancer patients than in patients with benign diseases (BALF: 6.75 ± 1.72 ng/mL vs. 1.91 ± 1.02 ng/mL; serum: 5.35 ± 1.27 ng/mL vs. 1.52 ± 1.06 ng/mL) ($p<0.0005$) [17]. Also, the current results agreed with Rong et al. who conducted a study to investigate the expression of annexin A1 in lung cancer patients and analysed the relationship with respect to the clinico-pathological features and assessed whether annexin A1 as a potential serum marker for lung cancer. The results showed that lung cancer tissues exhibited higher expression of annexin A1 than the normal tissues ($P < 0.05$) and the serum annexin A1 of lung cancer patients also exhibited higher level than control groups ($P < 0.05$).

Moreover, increased serum annexin A1 was significantly associated with the pathological grade and clinical stage of lung cancer patients ($P < 0.05$) [33].

These results agreed with those of Qiu et al.. In that study, Individual sera collected from 85 patients within 1 year before a diagnosis of lung cancer and 85 matched controls from the Carotene and Retinol Efficacy Trial (CARET) cohort were hybridized to individual microarrays. According to their results, cases of lung cancer significantly had elevated levels of mean annexin I autoantibody than the control group ($P=0.001$) [34]. Moreover, the current findings were in agreement with Almatroodi et al. who analyzed the BALF samples from individuals with and without primary lung adenocarcinoma using liquid chromatography-mass spectrometry. In that study, one thousand and one hundred proteins were identified, 33 of which were found to be consistently overexpressed in all lung adenocarcinoma samples compared to non-cancer controls. This result showed that annexin A1 was overexpressed in patients with lung cancer in comparison with noncancer controls [35].

Within the same line, as supporting evidence for the current findings, Fang and his colleagues performed a study to investigate the oncogenic role of ANXA1 in NSCLC cells in vitro. RNA interference was used to downregulate ANXA1 expression in A549 and H1299 cells using a small interfering RNA lentiviral vector. The results of the study showed that ANXA1 knockdown suppressed the proliferation, migration and invasion of NSCLC cells. The authors provided evidence suggesting that ANXA1 may contribute to the growth and invasion of NSCLC cell lines, and ANXA1 may be exploited as an in vitro therapeutic target for the treatment of NSCLC [36]. An explanation for the increased level was recently suggested by Allen and his colleagues who studied the vascular expression pattern of anxA1 in non–small-cell lung carcinoma (NSCLC). The authors isolated an antibody capable of binding N-terminal–truncated anxA127-346 and employed it in immunohistochemical studies of human lung specimens. Lung tumor specimens evaluated with this antibody revealed vascular (endothelial) anxA1 expression in five of eight tumor samples studied, but no vascular anxA1 expression was observed in normal lung tissue. Tumor microarray analysis further demonstrated positive vascular staining for anxA1 in 30 of 80 NSCLC samples, and positive staining of neoplastic cells was observed in 54 of 80 samples. No correlation was observed between vascular and parenchymal anxA1 expression. Two rodent tumor models, B16-F10 and Py230, were determined to have upregulated anxA1 expression in the intratumoral vasculature [10].

For biomarkers, optimum sensitivity and specificity are crucial. When the diagnosis of a disease is suspected, we need a marker with high specificity to help discern it. Meanwhile, its sensitivity should be such as to allow us to detect it at an early time. Our results were similar to Shepl et al. who showed that by receiver-operating characteristic curve (ROC curve) of annexin A1 in BALF and serum between malignant group and non-malignant group, the cut-off value between regards annexin A1 in BALF was 13 ng/ml, sensitivity 90%, specificity=90%, positive predictive value (PPV) =90, and negative predictive value (NPV)=90 with accuracy 90%. The cutoff as regards annexin A1 in serum was 12 ng/ml, sensitivity 85%, specificity=80%, PPV=81, and NPV=84 with accuracy

83%. BALF and serum annexin A1 in lung cancer had combined sensitivity and specificity of 95 and 90%, respectively, which were more than that of BALF and serum alone [32].

According to Biaoxue et al., the cutoff value of annexin A1 in BALF obtained by ROC analysis was 4 ng/mL. With this threshold, the sensitivity and specificity of annexin A1 in BALF were 94.2% and 90.2%, respectively. The calculated serum threshold was 3.1 ng/mL, with a sensitivity and specificity of 93.02% and 87.80%, respectively [17]. In the study conducted by Rong et al. by using receiver operator characteristic curve analysis, the cutoffs for distinguishing lung cancer from normal and benign groups were 4.77 and 4.84 ng/ml respectively. The sensitivities of annexin A1 for distinguishing lung cancer from normal and benign groups were 98.9% and 97.4%, and specificities were 88.3% and 66.4% [33].

In the current study, the serum and BAL Annexin A1 level didn't show any statistically significant correlation with the tested clinical and laboratory data in the malignant group. However, in the non-malignant group, there was a statistically significant positive correlation between BAL Annexin A1 level with age. No previous studies have commented on the correlation between serum and BAL Annexin A1 with the tested parameters like the current study. A limitation of our study is the case-control design. Without verification from medical records, we cannot exclude recall bias related to self-reported family history of cancer and non-malignant lung diseases. Furthermore, the small sample size and being a single center study were also other limitations of the current study that could decrease the power of the obtained results.

Conclusion

Based on the current findings, it could be revealed that males, smokers, and the elderly are more likely to develop lung cancer. In cases of lung cancer, mass and LNs occur more frequently. Both serum and BAL of annexin A1 could be used as sensitive markers in the diagnosis of lung cancer with higher accuracy in BAL annexin A1 levels.

Conflict of Interest

Authors declare no conflicts of Interest.

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