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Clinical value of interleukin - 6 as a potential biomarker for bronchogenic carcinoma

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Abstract
Background: Interleukin - 6 is found to be involved in various cancer types but countable studies has investigated its role in cancer lung.
Objectives: The purpose of this research was to assess Interleukin-6 levels in bronchoalveolar lavage (BAL) and serum of cases with cancer lung and cases with benign lung conditions, and to determine its diagnostic utility for cancer lung.
Subjects and Methods: This prospective interventional analytic study was conducted 40 cases at the Chest Department of Tanta University Hospitals. Cases were divided into two distinct groups: Group 1 comprised twenty individuals with bronchogenic carcinoma, while Group 2 comprised twenty individuals with non-malignant lung illnesses with established causes. All cases received a clinical examination, demographic information, and standard investigations. Using ELISA, the expression of interleukin-6 in BAL and serum was analysed.
Results: Interleukin-6 levels were elevated in the BAL and serum of cancer cases versus the control group. Interleukin-6 levels in BAL and serum were positively correlated with advanced clinical stages of cancer lung, as were Interleukin-6 levels in BAL and serum.
Conclusions: High BAL Interleukin-6 levels were associated with advanced clinical stage of cancer lung and correlated positively with serum Interleukin-6 levels. BAL Interleukin-6 was determined to be a molecular biomarker for cancer lung diagnosis and prognosis based on its high sensitivity and specificity in detecting lung carcinoma.

Keywords: Cancer lung, BAL, interleukin 6

Introduction
Worldwide, bronchogenic carcinoma is the main cause of cancer-related death [1]. Several studies have documented the significance of cytokines in bronchoalveolar lavage (BAL) for the diagnosis of lung cancer [2]. It indicates that interleukin 6 has an inhibitory effect on the anti-tumor immune response and promotes tumorigenesis via paracrine or autocrine mechanisms [3].

Patients and Methods
This prospective case control analytic research enrolled forty patients at Tanta University Hospitals' Chest Department. The duration of study was 18 months, started from December 2019 till June 2021. The ethics review board at Tanta University's School of Medicine gave their approval of the study's methodology.

Ethical consideration
- Written informed permission was acquired from all subjects before to enrollment in the study, along with an explanation of the significance of the research and the procedures that were initiated.
- Before beginning the study, approval from the Ethics Board of Tanta Faculty of Medicine was acquired.
- Confidentiality and individual privacy were observed at all stages of the research.
- Participants may withdraw from the study at any time without incurring any penalties.
- (Approval number 33290/08/19).

The cases were splitted into 2 groups: Group 1 comprised 20 subjects diagnosed by histopathological examination as bronchogenic carcinoma. They were (17) males and (3) females & their ages ranged from 45 to 70 with a mean of (58.75±6.71) years. The tumors were subtyped histologically and staged according to TNM staging system [4]. It included squamous cell carcinoma, adenocarcinoma and small cell carcinoma. The numbers of cases with squamous cell carcinoma, adenocarcinoma and small cell carcinoma were 9, 7 and 4 with percentage of 45%, 35% and 20% respectively.

Group II included 20 cases with non-malignant lung diseases with known etiologies (TB, pneumonia, lung abcess), they were (16) males and (4) females& their ages ranged from 40 to 75 with a mean of (55.00 ± 9.56) years. it included bronchopneumonia, lobar pneumonia, lung abcess, pulmonary TB. The numbers of bronchopneumonia, lobar pneumonia, lung abcess and pulmonary TB were 3, 6, 6 and 5 with percentage of 15%, 30%, 30% and 25% respectively.

Cases previously received chemotherapy or radiotherapy cases with multiple organ dysfunction or those who had a contraindication for bronchoscopy were excluded from the study [5].

Detailed history, complete clinical examination, chest x-ray, CT lung scan and pre-bronchoscopic investigations involving ECG, coagulation profile and spirometery were done for all cases.

BAL collection: Bronchoscopy was done using PENTAX bronchoscope EB012780, 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, collection trap were prepared. Cases at risk of bronchospasm were premedicated with bronchodilators. 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 ml of saline was poured with a syringe. Suction was avoided until irrigation distillarion was completed and gentle suction (50-80mmHg) was applied. Then, the fluid specimens were collected in the collection trap. Steps 3,4 and 5 were repeated, up to 5 times to obtain an adequate specimen (40-60 mL). Patient was observed and monitored for about 1 hour after the procedure [6].

Preparation of BAL sample

BAL samples were collected in a siliconized container. The fluid was filtered and centrifugation was done at 4 °C- 3000 rpm for thirty minutes to separate the floating impurities. The samples were numbered and stored at -20 °C.

Serum sample preparation: After collecting the sample of blood, it clotted by leaving it constant at room temperature for 10-20 minutes. Then, the sample was centrifuged for 20 minutes and the serum was separated in eppendorf tube and stored at -20 C.

Interluekin- 6 Detection

Serum and BAL annexin A1 of studied groups was measured using commercially available ELISA (Enzyme linked immunosorbentassay) Kit supplied by Sun Red technology co., Germany according to manufacturer instructions.

Statistical analysis

Statistical presentation and analysis of this study was done using the mean, standard deviation and chi-square test by SPSS V.22 (Statistical Package for social sciences, version 22, IBM).

Results

Statistical comparison between both studied groups regarding age, sex, smoking status and smoking index showed that no significant statistical difference was present between both studied groups [Table 1].

Table 1: Demographic data and smoking status of the studied groups

			Group 1	Group 2	Test	p-value
Demographic data	Age	Range	45 – 70	40 – 75	T: 1.435	0.159
		Mean ± S. D	58.75 ± 6.71	55.00 ± 9.56		
	Sex	Male (%)	17 (85%)	16 (80%)	X^2: 0.173	0.677
		Female (%)	3 (15%)	4 (20%)		
Smoking status	Smoking (p/y)	Smoker (%)	11 (55%)	10 (50%)	X2: 0.559	0.756
		Ex smoker (%)	5 (25%)	4 (20%)		
		Non Smoker (%)	4 (20%)	6 (35%)		
	Smoking index	Range	300 – 800	400 – 800	T: 1.407	0.176
		Mean ± S. D	520.45 ± 171.33	525.0 ± 168.74		

Significant p. value >0.5* X^2: Chi- square test*
S.D: standard deviation* T. test: student ś test*

Statistical comparison between both studied groups regarding Interleukin 6 in serum and in BAL [Table 2] showed that the mean values were significantly elevated in group I (P value = 0.001, 0.001 respectively).

Table 2: Mean level of Interleukin 6 levels in BAL and serum in two studied groups

		Range	Mean ±SD	t-test	p-value
Interleukin 6 in BAL (pg/ml)	Group I	90.7 – 215	138.06 ± 35.43	9.951	0.001*
	Group II	30 – 102	47.93 ± 19.64		
Interleukin 6 in serum (pg/ml)	Group I	88 – 200	128.48 ± 34.94	9.351	0.001*
	Group II	30 – 96	46.36 ± 17.93		

*BAL: Bronchoalveolar lavage

The correlation was significantly positive between level of BAL and serum Interleukin 6 and advanced clinical stages of cancer lung in group I (p. value 0.0 1, 0.002) respectively. [Table 3]

Table 3: BAL and serum interleukin 6 in different clinical stages of cancer lung in group I

		Range			Mean	±	S. D	f-test	p-value
Interleukin 6 in BAL (pg/ml)	Limited	160	–	200	185.33	±	22.03	7.127	0.001*
	Extensive	215	–	215	215.00	±	0.0		
Interleukin 6 in serum (pg/ml)	Limited	156.5	–	191	177.50	±	18.43	6.821	0.002*
	Extensive	200	–	200	200.00	±	0.0		

The correlation was significantly positive between Interleukin- 6 level in BAL and serum of cancer lung cases (p-value 0.001). [Table 4]

Table 4: Correlation between interleukin 6 levels in BAL and serum in cancer lung cases

	Interleukin 6 in serum (pg/ml)	
	r	p
Interleukin 6 in BAL (Pg/ml)	0.990	0.001*

Receiver operating characteristic curve (ROC curve) of IL-6 in BAL and serum between group I & II: (Table 5, figure 1, 2). The cut off value between group I & II as regards interleukin 6 in BAL was 95 ng/ml, sensitivity 95%, specificity 90%, positive predictive value (PPV) 90, negative predictive value (NPV) 95, with accuracy 92.5%. The cut off value between group I & II as regards interleukin 6 in serum was 90 ng/ml, sensitivity 90%, specificity 90%, PPV 90, NPV 90, with accuracy 90%.

Table 5: ROC curve of BAL and serum annexin A1 between two groups

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Interleukin 6 in BAL (pg/ml)	95	0.993	95	90	90	95	92.5
Interleukin 6in serum (pg/ml)	90	0.988	90	90	90	90	90

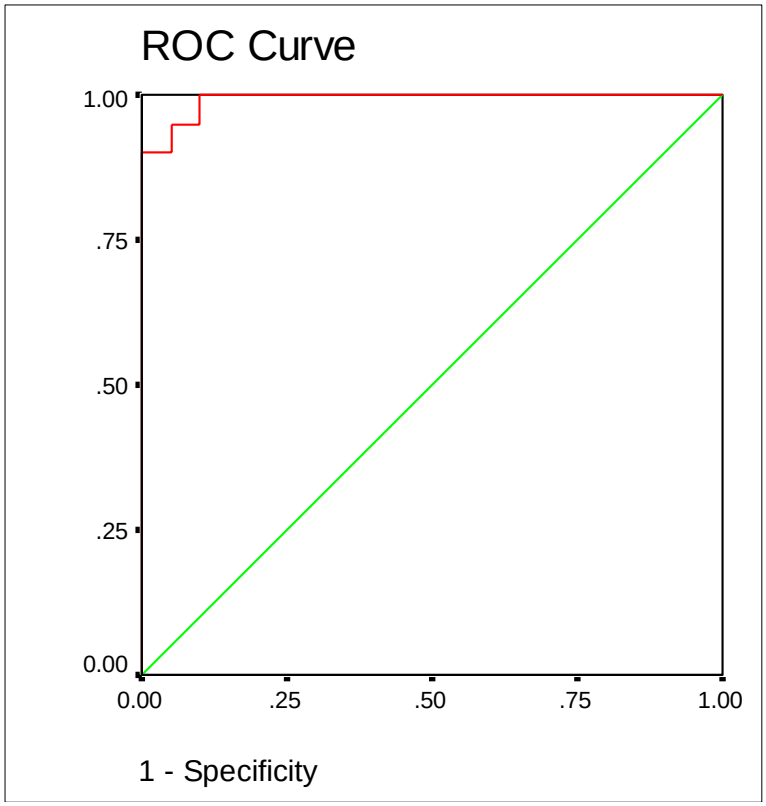

Fig 1: ROC curve of Interleukin 6 (pg/ml) in BAL between the two studied groups

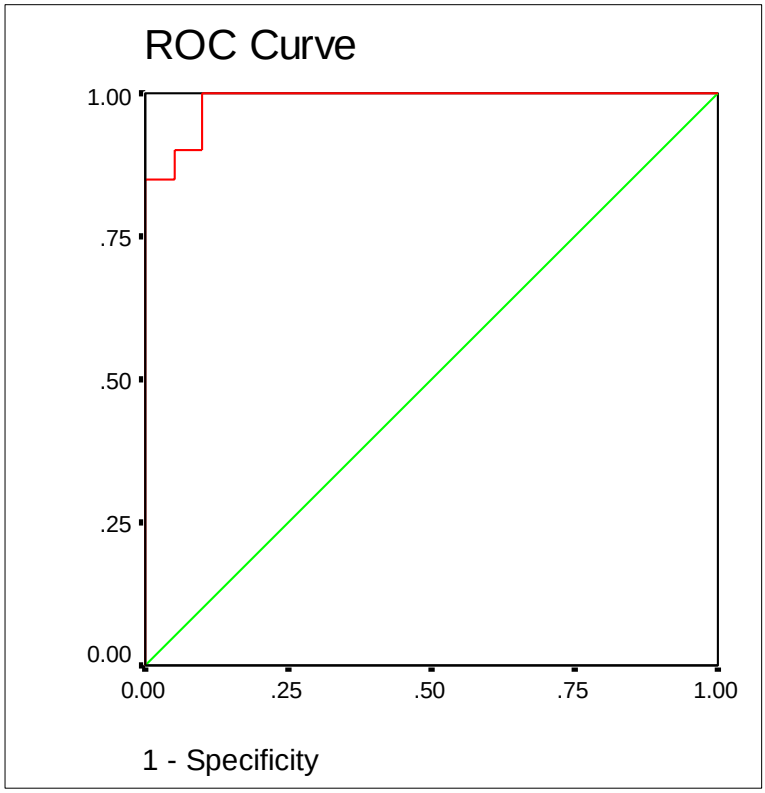

Fig 2: ROC curve of interleukin 6 (pg/ml) in serum between the two studied groups

Discussion

Cancer lung is the leading cause of cancer-related deaths worldwide [7]. Interluekin-6 is a potent multifunctional protein. Monocytes and macrophages are predominantly responsible for interleukin 6 production. interleukin 6 takes part in the induction of B-lymphocytes to immunoglobulin synthesis, the activation and differentiation of T-cells and in the activation of megakaryocytes [8\.

In our study, the mean value of BAL interleukin 6 level was significantly raised in cancer lung (P value = 0.001). This result agrees with Domagala-Kulawik. 2020 [9] who reported increased levels of BAL fluid interleukin 6 in cases with cancer lung versus the control group (P value = 0.007).

The mean value of serum interleukin 6 level was significantly elevated in cancer lung (P value = 0.001). This result agrees with Kasprzyk *et al*. 2006 [10] who found that interleukin 6 has been found strongly elevated in cancer lung cases.

In our study we cleared that the mean level of interleukin 6 in serum in group I was significantly raised in smokers than non-smokers (p-value 0.013). This result agrees with Bradford *et al*. 2017 [11] who found that interleukin 6 was significantly raised in smoker versus non-smokers, (P value = 0.0009).

In our study we illustrated that the mean value of IL- 6 was significantly raised in small cell cancer lung than non-small cell carcinoma (p-value 0.001).

Our results were coincided with study by Ujiie *et al*. 2012 [12] who found that the interleukin 6 levels were significantly raised in cases with SCLC.

In our study, we found that there was a significant positive correlation between interleukin 6 in serum and advanced clinical stages of cancer lung (p. value 0.002). Our data agree with the results of Songür *et al*. 2004 [13] who found that the mean value of interleukin 6 was raised in stage IV compared with stage I cancer lung.

In this study we illustrated that there was a positive correlation between Interleukin 6 levels in BAL and serum of cancer lung cases (p-value 0.001). Our data agreed with the results of Chen *et al*. 2014 [14] who found that there was a significant positive correlation between interleukin 6 in BAL and serum of cancer lung (P. value 0.003).

ROC analysis of BAL interleukin 6 between both studied groups in this study showed a cut off value 95 ng/ml with sensitivity 95%, specificity 90%, PPV 90, NPV 95 with accuracy 92.5%. These results agree with Chen *et al*. 2014 [14]. ROC analysis revealed that cut off value of BAL interleukin 6 was 10.85 pg/ml with sensitivity 62.2%, specificity60.6%.

ROC analysis of serum interleukin 6 between both studied groups in this study showed a cut off value 90 ng/ml with sensitivity 90%, specificity90%, PPV 90, NPV 90 with accuracy 90%. These results agree with Silva *et al*. 2017 [15]. ROC analysis revealed that cut off value of BAL interleukin 6 was 8.05 pg/ml with sensitivity 86.8%, specificity 43.6%.

Conclusion

It is concluded that The BAL interleukin 6 expression is upregulated in cancer lung cases. Increased BAL interleukin 6 level correlates with advanced clinical stages of cancer lung. BAL interleukin 6 level is positively correlated with serum interleukin 6 in cancer lung cases. High sensitivity and specificity of BAL interleukin 6 for detection of cancer lung indicated that it could be considered a molecular marker for early diagnosis of pulmonary carcinoma.

Limitations

The limitations of the present research was the small number of cases. The duration of this study was short and limited. The study lacked immunohistochemistry to measure the level of interleukin 6 in cancer lung and benign lung diseases tissues.

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