Diagnosis of Pulmonary Tuberculosis by measurement of ADA, CEA and PCR in Bronchoalveolar lavage and compare with smear and culture of BK in the patient with pulmonary infiltration

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Abstract

Background: Pulmonary tuberculosis (TB) is one of the problems in diagnosis and management of patients with pneumonia and pleural effusion. Bronchoscopy and bronchoalveolar lavage (BAL) can help us in the differential diagnosis. Tumor markers are substances that are produced by cancer or other cells of the body in response to diseases. These substances can be found in the blood, urine, or body fluids, for example in bronchoalveolar lavage of some patients with pulmonary diseases. CEA is a tumor marker that can help us for diagnosis and monitoring of peripheral lung cancer. ADA has been shown to rise in the BAL of patients with TB. Because the sputum smear negative TB patients have been a diagnostic challenge for health professionals this study was designed to determine the diagnostic value of ADA & CEA & PCR in BAL fluid and then compare the relationship of these markers with sputum and clinical finding in diagnosis of pulmonary tuberculosis.

Material and Method: A cross sectional study conducted in Shahrekord Hajar hospital where 100 patients were enrolled in our study (62 females, 38 males). These patients were admitted to hospital and bronchoscopy with BAL was performed, then fluid was analysed for ADA,CEA , PCR and sputum smear for TB.

Result: From 100 patients who were evaluated in this study 9 patients had TB, 39 patients had pneumonia, 11 patients had primary lung malignancy, 9 patients had metastases to lung and 8 patients had bronchitis. In Patients with TB the level of ADA was not significantly elevated in BAL. ADA increased significantly in patients with pneumonia (P: 0/95). In patients with metastases to lung and primary lung cancer and a combination of both groups compared to other patients without malignancy there was no relationship between CEA. PCR was positive in TB and other patients, but significantly increased in TB. (p=0/021)

Discussion: Although it was thought that CEA may be elevated in BAL of patients with malignancy and ADA in tuberculosis we did not find a correlation in these patients. This study showed that ADA and CEA levels in BAL fluid does not help us in diagnosis of TB or cancer. It may have afalse positive in pneumonia, COPD and other diseases, but PCR for TB may assist in TB diagnosis but not alone.

Key words: Bronchoalveolar lavage (BAL), adenosine deaminase (ADA), polymerase chain reaction (PCR), Bronchoscopy. Carcino embryonic antigen (CEA). Tuberculosis (TB)

Introduction

CEA is a glycoprotein weight approximately 200,000 dalton. CEA is mainly found in the fetal gastrointestinal tract and in fetal serum (1,2). Secretion of CEA decreases after birth, slight to moderate CEA elevation occurs in benign situations such as in lungs, pancreas, liver disease or in malignant disease in colorectal cancer(3). Some studies have demonstrated CEA increase in BAL fluid in lung cancer, IPF COPD, and pneumonia. Applications are diagnostic but it is useful for monitoring recurrence of malignancy and prognostic factors(4).

ADA is a key enzyme in purine metabolism (5). ADA is a hydrolytic catalyzer enzyme that is irreversibly responsible for conversion of inosine to adenosine (6). ADA help in differentiation of tissues particularly height in lymphocytes in humans (7). ADA can be increased in TB effusion.

TB is the second infective etiology in mortality in the world, but there are many problems in diagnosis of TB, particularly negative smear TB.

Lung cancer is an etiology for mortality for many humans and many humans suffer from lung cancer. It has the highest mortality after prostate, breast, and colon cancer. Studies show CEA in BAL fluid is increased. We have problem in diagnosing peripheral lung cancer, because bronchoscopy can have accuracy of approximately 48%-80% in peripheral lung cancer and 79%-95% in central lung cancer.

Bronchoscopy is a method that is used to diagnose various pulmonary diseases. It enters from the mouth or nose to arrive at lung (8).

PCR is a rapid technique for replication of DNA and it is fast method for diagnosing TB but it is not cost beneficial. It used to amplify a few copies or many copies of strands of DNA across several ordersof generating thousands and millions of copies of a particular sequence of DNA(10).

A few studies have evaluated BAL fluid ADA alone for diagnosis and CEA alone for diagnosis of TB or lung malignancy, but we evaluated this markers for various pulmonary diseases. This study investigated ADA , CEA , and PCR for TB and malignancy in BAL fluid.

Material and Methods

A cross sectional study was performed then at the Hajar Shahrekord hospital in Iran between 2013-2015.

100 patients with various respiratory symptoms admitted to the hospital, who had indication for bronchoscopy and bronchalveular lavage were includes in the study; exclusion criteria were as follows (11):

1) They have contraindication such as hypoxemia, MI, recent angina, respiratory failure, incompatibility patient, tracheal obstruction

2) history of alcohol abuse, known case of malignant liver disease

Bronchoscopy and BAL was performed then samples sent to laboratory for evaluation of CEA, ADA levels and PCR in BAL fluid were compared to each other by ADA and CEA kit.

ADA investigated by ADLS2210 (ADA liquid stat) Reagent kit. CEA was evaluated by the sandwich principle and Modular analytics E170 kit.

We followed patients and did diagnostic evaluation as required (CXR,CT scan. bronchoscopy, AFB) for diagnosis of pulmonary disease, then patients were divided into six groups. The aim of this study was to investigate the relationship between ADA, CEA, and PCR in BAL and pulmonary tuberculosis and lung cancer.

SPSS16 was used for the analytical statistics of the data.

Results

From 100 patients that enrolled into the study 63 were female and 27 wer male with minimum age of 13, and maximum age of 84. Mean, median, Standard-deviation of age respectively was 57, 53, 19. For ADA the Mean, medium, and standard deviation was respectively 4, 81, 4, 1, 66 and for CEA was 11, 12, 4, 93, 16, 3. Minimum level for ADA and CEA was 3, 0/9 and maximum was 8, 56/6 .

We regarded normal cut off point for ADA as <3/5, and for CEA <5 for nonsmoking and CEA <10 for smoking patients. We divided patients into 6 groups : 1) 6 patients had TB . 2) 39 pneumonia 3) 11 primary lung cancer. 4) 9 metastases to lung. 5) 8 chronic bronchitis. 6)27 other patient with cardiac and respiratory disease

We measured level of ADA and CEA in BAL of 100 patients, then we processed the results.

The prevalence of symptoms in these patients was dyspnea (67), dry cough (31), productive cough (26), dysphonia (1), fever(11), chest pain (5) .We compared patients based on symptoms of the various pulmonary diseases that are shown in Table 1.

We used Smirnoff test because there was no normal distribution for ADA, CEA. Mann Whitney test ADA in TB group (9) and other groups (91) were not significantly different (P=0/14), primary lung malignancy(11)(P=0/19), metastases to lung (9)(P=0/16), combination two group metastases with primary cancer (20)(P=0/73),chronic bronchitis (8)(P=0/93).

We found ADA in pneumonia significantly different from the other groups, (P=0/83). It is shown that ADA can be elevated by pneumonia (infection of lung parenchyma).

Table 1: Prevalence of clinical symptoms patient separation of clinical diagnosis

	No.	DRY COUGH		DYSPNEA		PRODUCTIVE COUGH		HEMOMTESIA		FEVER		DYSPHONIA		CHEST PAIN	
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
TB	9	5 (55.6%)	4 (44.4%)	5 (55.6%)	4 (44.4%)	2 (22.2%)	7 (78.8%)	1 (11.1%)	8 (88.9%)	2 (22.2%)	7 (78.8%)	0	9 (100%)	0	9 (%100)
Primary Lung Cancer	11	0	11 (100%)	9 (81.8%)	2 (18.2%)	1 (9.1%)	10 (90.9%)	0	11 (100%)	0	11 (100%)	0	11 (100%)	2 (18.2%)	9 (81.8%)
Metastases	9	5 (55.6%)	4 (44.4%)	6 (66.7%)	3 (33.3%)	2 (22.2%)	7 (78.8%)	0	9 (100%)	2 (22.2%)	7 (78.8%)	0	9 (100%)	1 (11.1%)	8 (81.8%)
Metastases and lung cancer	20	5 (25%)	15 (75%)	15 (75%)	5 (15%)	3 (15%)	17 (75%)	0	20 (100%)	2 (10%)	18 (90%)	0	20 (100%)	3 (15%)	17 (75%)
Bronchitis	8	1 (12.5%)	7 (87.5%)	6 (75%)	2 (25%)	4 (50%)	4 (50%)	2 (25%)	6 (75%)	0	8 (100%)	0	8 (100%)	1 (12.5%)	7 (87.5%)
Pneumonia	39	14 (35.9%)	25 (64.1%)	23 (59%)	16 (41%)	12 (30.8%)	27 (69.2%)	5 (12.8%)	34 (87.2%)	34 (87.2%)	33 (84.6%)	0	39 (100%)	1 (2.6%)	38 (97.4%)

Results showed 86 patients had negative PCR and 14 patients with positive PCR. In the TB (9) group, 4 patients had positive PCR and 5 negative PCR, in the other groups (91) 10 patients had positive PCR and 81 patients negative PCR. This finding shows a significant relationship between TB and positive PCR (P=0/021).

Positive PCR or increasing ADA more than cut of point (>3/5) was only found in 5 patients including 1 patient compared to (9) patient with a known case of TB and with this two tests 4 patient without TB. It showed there was no significant difference between TB and the other groups so we cannot use them only for diagnosis of TB.

Measurement of CEA in BAL showed there was not significant difference between patients with primary lung malignancy (P=0/78), metastases to lung (p =0/25), and combination of both (p=0/54) with other groups including TB (p=0/63), pneumonia (P=0/83),and bronchitis (P=0/45).

Our results including mean, medium, standard deviation of CEA, ADA have shown the breakdown of diagnoses.

Discussion

The finding showed a significant difference in ADA levels, in pneumonia groups compared to other groups, but there was no relationship between TB and the other groups in ADA level BAL fluid. In the study of Reechaitichikal et al, that compared ADA levels in BAL fluid of pulmonary TB, lung cancer, and other pulmonary disease they did not find significant difference among these groups (P=0/56)(7) but Banish et al's study showed ADA level as a significant difference in TB patients compared to non TB pulmonary disease and control group (12). In our result there was demonstrated positive PCR in non TB disease, but it had significant different between TB and non TB groups (P=0/021). Boonsarangsuk et al showed ADA activity of TB cases was significantly different from that of other patients with pulmonary disease (p<0/001). To differentiate TB from solid tumor the AUC was significantly higher for combination of ADA activity >3 Ana TB PCR (0/7) than for ADA alone (p<0/001) ,or for TB PCR alone (p<0/001); it showed sensitivity of 72% in combination of the two tests but alone sensitivity for ADA was 58/7% and PCR sensitivity alone was 28/1% (13).

Table 2: Median, mean, standard deviation, P. value, minimum and maximum data rate of the ADA in lung disease

		Median	Mean	Standard deviation	P. Value	Minimum	Maximum
TB	Yes	3/6	3	2	0/4	1/4	8
	No	4/86	4	4/32			
PRIMARY LUNG CANCER	Yes	4/81	4	1/66	0/19	3	8
	No	4/74	4	4/39			
METASTASES	Yes	8	4	10/9	0/16	3	37
	No	4/32	4	2/73			
METASTASES & LUNG CANCER	Yes	6/25	4	7/36	0/73	3	37
	No	4/37	4	2/85			
BRONCHITIS	Yes	4	4	1/19	0/93	2	6
	No	4/81	4	4/34			
PNEUMONIA	Yes	4/03	4	1/9	0/95	0/7	11
	No	5/21	4	5/09			

This study investigating CEA in BAL did not increase significantly in primary lung cancer, metastases to lung or both combinations. Aytemur Salak et al demonstrated CEA levels in BAL fluid do not differ between lung cancer and other groups, sensitivity, specificity of CEA in BAL was respectively 81/8%,45/1%. Diego et al investigated CEA level in serum and BAL in lung cancer patients and pneumonia and healthy patients; CEA in BAL was 5/1. It showed CEA level in BAL with respective sensitivity and specificity 77%-94% can help to us for diagnose of lung cancer (14).

However there was a high number of T-lymphocytes in BAL related to ADA, specially ADA (2) , but different studies showed different results on ADA level on BAL for diagnosing Tb. It could be due to geographic location, low and high incidence regions, or the method of measurement in different location; any way ADA cannot help to diagnose Tb.

Diagram 1: Comparison of ADA's total and average of lung disease to diagnosis

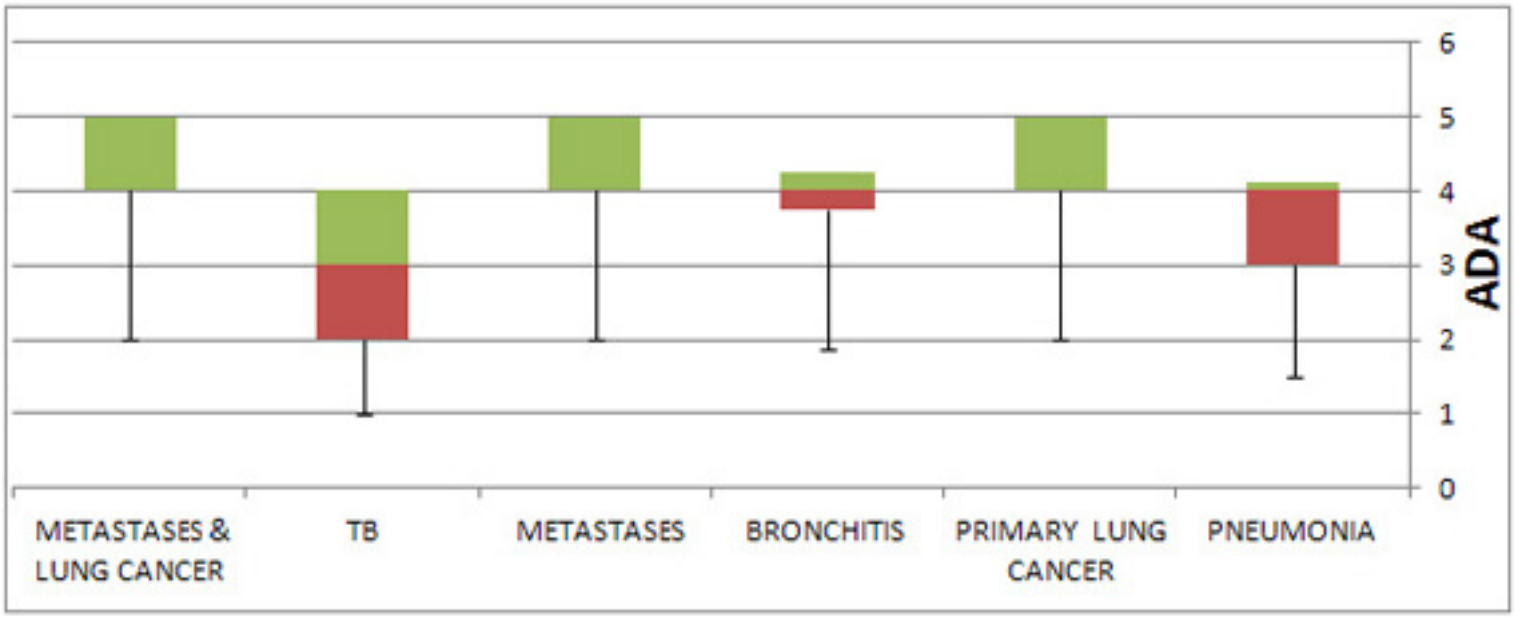
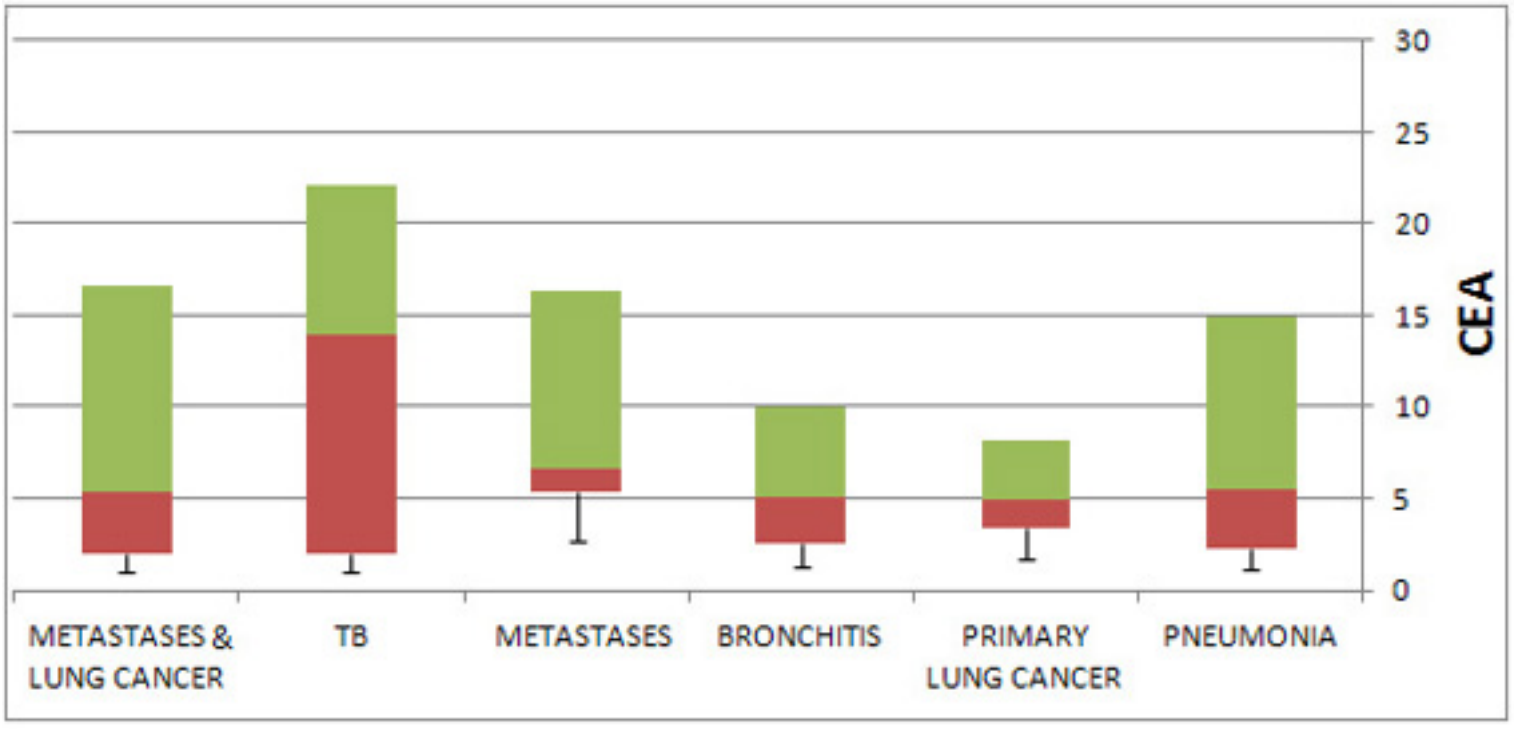


Table 3: Median, mean, standard deviation, P. Value, minimum and maximum data rate of the CEA in lung disease

		Median	Mean	Standard deviation	P. Value	Minimum	Maximum
TB	Yes	11/3	11/3	11/06	0/68	1/12	35/4
	No	11/26	11/26	1/48			
PRIMARY LUNG CANCER	Yes	11/12	11/12	16/3	0/78	0/9	56/6
	No	11/22	11/22	1/43			
METASTASES	Yes	14/51	14/51	12/6	0/25	1/26	36/9
	No	10/88	10/88	1/46			
METASTASES & LUNG CANCER	Yes	12/64	12/64	14/48	0/54	0/9	56/6
	No	10/85	10/85	1/45			
BRONCHITIS	Yes	8/18	8/18	9/05	0/52	0/5	28/16
	No	11/47	11/47	1/48			
PNEUMONIA	Yes	11/22	11/22	13/66	0/83	0/5	69/86
	No	11/2	11/2	1/5			

Diagram 2: Compare CEA's total and average of lung disease to diagnosis



Conclusion

This study showed that ADA levels in BAL fluid do not help us in TB diagnosis. It may be that it has false positive in pneumonia, compared with other disease, but PCR for TB may help us for TB diagnosis but nt alone. CEA level in BAL cannot differentiate lung malignancy.

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