

DIAGNOSTIC UTILITY OF ADENOSINE DEAMINASE IN SERUM AND BRONCHOALVEOLAR LAVAGE FLUID FOR SCREENING LUNG CANCER IN WESTERN IRAN

DIJAGNOSTIČKA KORIST ADENOZIN-DEAMINAZE U SERUMU I BRONHOALVEOLARNOM LAVAŽU ZA SKRINING RAKA PLUĆA U ZAPADNOM IRANU

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Summary

Background: This study aimed to determine adenosine deaminase (ADA) activity as a possible screening tool in lung cancer patients.

Methods: Blood samples were collected from 30 subjects with positive pathological tests and 62 patients with negative pathological tests as a control group. The enzymatic activity of total ADA and its isoenzymes was determined.

Results: tADA and ADA2 isoenzyme activity was significantly higher in cancerous patients compared to benign controls in serum and BAL fluid. Using a cut-off level of respectively 35.22 U/L and 31.80 U/L for BAL total ADA and ADA2, sensitivity and specificity were 100% and 81% for total ADA and 95% and 98% for ADA2.

Conclusions: Adenosine deaminase may play important roles in the pathophysiology of lung cancer and because of its

Kratak sadržaj

Uvod: Cilj ove studije bio je da se odredi aktivnost adenzin-deaminaze (ADA) kao potencijalnog parametra za skrining kod obolelih od raka pluća.

Metode: Uzorci krvi uzeti su od 30 subjekata sa pozitivnim patološkim testovima i 62 pacijenta sa negativnim patološkim testovima koji su činili kontrolnu grupu. Određena je enzimaska aktivnost ukupne ADA (tADA) i njenih izoenzima.

Rezultati: Aktivnost tADA i izoenzima ADA2 bila je značajno povišena kod pacijenata sa kancerom u poređenju sa benignim kontrolama u serumu i bronhoalveolarnom lavažu (BAL). Uz cut-off vrednosti od 35,22 U/L, odnosno 31,80 U/L za ukupnu ADA i ADA 2 u BAL, osetljivost i specifičnost bile su 100% i 81% za tADA, odnosno 95% i 98% za ADA2.

Zaključak: Adenzin deaminaza može igrati važne uloge u patofiziologiji raka pluća i može se zahvaljujući svojoj moći

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might be considered as a useful screening tool among the other markers in lung cancer diagnosis.

Keywords: adenosine deaminase, lung cancer, serum, bronchoalveolar lavage, diagnostic value

Introduction

Lung cancer is the most frequent cancer and the most important cause of cancer-related death in men. About one to two million people die from lung cancer every year worldwide. Lung cancer causes more deaths than the other three most common cancers combined, i.e., colon, breast and prostate (1, 2). In an attempt to describe human lung cancer, many studies have concentrated on enzyme activities (3–5). In addition, some studies established alteration of enzyme activity in bronchoalveolar lavage (6, 7). To better understand purine enzymology in lung cancer, more attention has been paid to the investigation of the interrelationship among the carcinogenic process and enzyme activity (*de novo* and salvage pathways) for purine biosynthesis (3, 4).

Adenosine Deaminase (ADA) is a hydrolytic enzyme in the purine metabolism pathway that catalyses the conversion of adenosine to inosine (8). ADA is widely distributed in human tissues and involved in immune system development. Two major isoforms of ADA have been isolated with different characteristics (8, 9). Alteration in serum ADA activity has been reported in a broad range of lung diseases such as tuberculosis and chronic obstructive pulmonary disease (COPD) (10, 11). ADA is known as a marker of T-lymphocyte activation (12). ADA levels are found to be increased in effusions related with tuberculosis. Measurement of ADA in clinical practice is frequently considered as a noninvasive diagnostic test for tuberculosis with 90–100% sensitivity and 89–100% specificity (4, 7, 13). Furthermore, it has been shown in several studies that ADA activities may be useful both in the diagnosis and monitoring of some malignancies (7, 14).

Previous studies have shown an inconclusive result for serum ADA activity in lung cancer (15–18). In addition, serum tumour markers are not useful in lung cancer diagnosis (1). The diagnosis of lung cancer is based on the histological and cytological examination of material usually obtained by bronchoscopy (endobronchial biopsies, washings, and brushings) (19). However, despite the high sensitivity of this technique in detecting tumours, in some cases false negative results can be seen (20).

The source of bronchogenic carcinoma is the bronchial epithelium (19). In addition, ADA production by neoplastic cells is increased (4, 7) and it could be assumed that respiratory secretions from lung cancer patients may contain high levels of ADA. So far,

smatrati korisnim parametrom za skrining, uz ostale markere u dijagnozi raka pluća.

Ključne reči: adenzin deaminaza, rak pluća, serum, bronhoalveolarni lavaž, dijagnostička korist

however, the measurement of ADA in bronchoalveolar lavage (BAL) and bronchial washings has proved unhelpful (16). Therefore, regardless of histological type and staging, for the investigation of ADA utility in the screening of lung cancer, this study aimed to evaluate the alterations in serum total ADA levels as well as ADA1 and ADA2 isoenzyme activities and the correlation between ADA activity in serum and BAL of lung cancer patients compared to control subjects.

Materials and Methods

Subjects

In this cross-sectional study, the population consisted of 92 patients who were admitted to Tohid hospital in Sanandaj (Kurdistan, Iran) because of suspicious pulmonary masses during January 2010 to February 2012, divided into two groups: patients with previously untreated lung cancer ($n=30$) and patients with benign lung disease ($n=62$). Diagnosis of malignancy was identified by histopathology of the lung tissue and/or cytology of bronchoalveolar lavage. Control groups comprised patients with benign lung disease (negative pathological tests). Written informed consent for participation was obtained and the project was approved by the Research Ethics Committee of Kurdistan University of Medical Sciences, Iran. Patients with a history of other malignancies as well as those with respiratory failure, in addition to patients with a history of alcohol abuse, known causes of liver disease, diabetes mellitus, tuberculosis, renal, liver or gastrointestinal disease and metastatic lung lesions were excluded from the study. Patients, aged 54–88 years (68.59 ± 9.65 years), and control groups were age-matched (45–87 years (62.4 ± 12.5 years)). All patients and control individuals were from Kurdistan, a province in western Iran with a population that is Kurdish. All patients included in the study had untreated bronchogenic carcinoma.

Sample collection and analysis

Fasting blood samples were collected; serums were separated, and stored at -70°C pending assay. Bronchoalveolar lavage was performed in the most affected lobe in localized disease. Three aliquots of 50 mL of normal saline solution at room temperature were infused and immediately aspirated using a syringe. Bronchoalveolar lavage (BAL) and serum was analyzed for ADA activity.

Determination of adenosine deaminase activity

Adenosine and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) were obtained from Sigma-Aldrich (Sigma, Missouri, USA). Serum total adenosine deaminase activity (ADA) was measured colorimetrically according to the Giusti method (21). For the determination of serum ADA2 isoenzyme activity, serum ADA1 activity was inhibited by the addition of EHNA into the serum samples (21, 22). Briefly, based on the Bertholet reaction, the absorption of colored indophenol complex formed from ammonia liberated from adenosine was quantified spectrophotometrically at 630 nm. To control the presence of ammonium before addition of exogenous adenosine, untreated samples were run in parallel. Estimated ADA1 activity was calculated by subtracting the ADA2 and ADAT activities using the inhibition of ADA1 by EHNA. Finally, all activities were expressed in unit per liter (U/L). Coefficient of variation (%CV) for this method is about 3%; it has a high reproducibility and very inexpensive materials are used (9). Giusti method needs to be done at 37 °C. Ammonia is the main interfering agent in this method. For removing ammonia effects in phosphate buffer and serum, sample blank and reagent blank tubes should be used. Phosphate buffer pH should be set to 6.2–6.8 and absorbance of reagent blank tube against demineralized water must be less than 0.03 (21).

Statistical analysis

Statistical analysis was carried out using SPSS 16 (SPSS Inc., Chicago) and because of the lack of normal distribution among variables (Kolmogorov-Smirnov test) non-parametric tests were used for analysis of data. Mann-Whitney test was used to analyze the difference between groups. P-values <0.05 were considered significant. Results were presented as Median. Receiver operating characteristic (ROC) curves were constructed to establish a sensitivity–specificity relationship. Cut-off values that provided the best combination of sensitivity and specificity were determined by ROC curve analysis. Sensitivity (true-positive/true-positive + false-negative), specificity (true-negative/true-negative + false-positive), positive predictive value (PPV, true-positive/true-positive + false-positive), negative predictive value (NPV, true-negative/true-negative + false-negative), positive likelihood ratio (LR+, sensitivity / 1-specificity) and negative likelihood ratio (LR-, 1-sensitivity / specificity) were calculated. Accuracy was defined as (true-positive + true-negative) / (true-positive + false-positive + true-negative + false-negative).

In addition, linear regression analysis was performed and means of the Pearson's Correlation Coefficient (r) were determined to show the correlation between tumour marker levels in serum and BAL fluids.

Results

Of the 92 studied persons, 30 (32.61%) were established to have a malignant and 62 (67.39%) were diagnosed to have a benign tumour. Of the 30 malignant cases, 29 (96.67%) were male and 1 (3.33%) female. Of the 62 benign cases, 56 (90.32%) were male and six (9.68%) female. In the malignancies group, 22 were squamous cell carcinoma, one adenocarcinoma, six small cell carcinoma and one large cell carcinoma. In the control group, thirty-one patients had low stages of chronic obstructive pulmonary disease (COPD- Stage I–II GOLD), 10 were chronic bronchitis, eight were pneumonia, eight were sarcoidosis and five were other diseases.

Stage I–IIIA disease was confirmed in three patients, whereas stage IIIB–IV disease was diagnosed in 27 patients according to the TNM staging. Of the malignant cases, fifteen patients (50%) were current smokers, five (16.67%) were ex-smokers and 10 (33.33%) were non-smokers. In the benign group, forty patients (64.52%) were current smokers, 10 (16.13%) were smoking abstinence for more than one year and 12 (19.35%) were non-smokers.

Table I shows tADA and its isoenzymes activity in the serum and BAL fluid of malignant and benign groups. Both serum and BAL total adenosine deaminase activity, regardless of the histological type, considerably increased in cancerous patients compared to the benign group (p value <0.001). A comparable shift was also observed in ADA2 isoenzyme activity in the studied groups, so that ADA2 was significantly increased in cancerous patients compared to benign group (p value <0.001, Table I). However, there was no significant difference for ADA1 activity between the studied groups. Linear regression analysis showed a noticeable direct correlation between tADA ($R^2=0.421$, $p<0.05$) and ADA2 isoenzyme ($R^2=0.257$, $p<0.05$) activity in serum and BAL fluid (Figure 1), whereas the correlation of ADA1 activity was not as strong ($R^2=0.175$, $p<0.05$) as that observed for ADA2 isoenzyme (data not shown). Linear regression analysis also revealed that total

Table I Median level of adenosine deaminase and its isoenzymes activity in serum and BAL of lung cancer patients and controls ($p<0.05$).

Sample	Group	tADA (U/L)	ADA1 (U/L)	ADA2 (U/L)
Serum	Lung cancer	29.40 (20.53–48.84)	6.81 (1.43–19.77)	24.69 (13.40–40.75)
	Benign group	17.97 (4.89–39.38)	4.42 (0.76–14.69)	12.6 (3.62–25.11)
BAL	Lung cancer	50 (35.22–165.18)	10.20 (1.39–20.54)	40.00 (23.80–144.64)
	Benign group	24.39 (13.16–47.26)	8.56 (0.0–23.89)	15.51 (0.0–33.49)

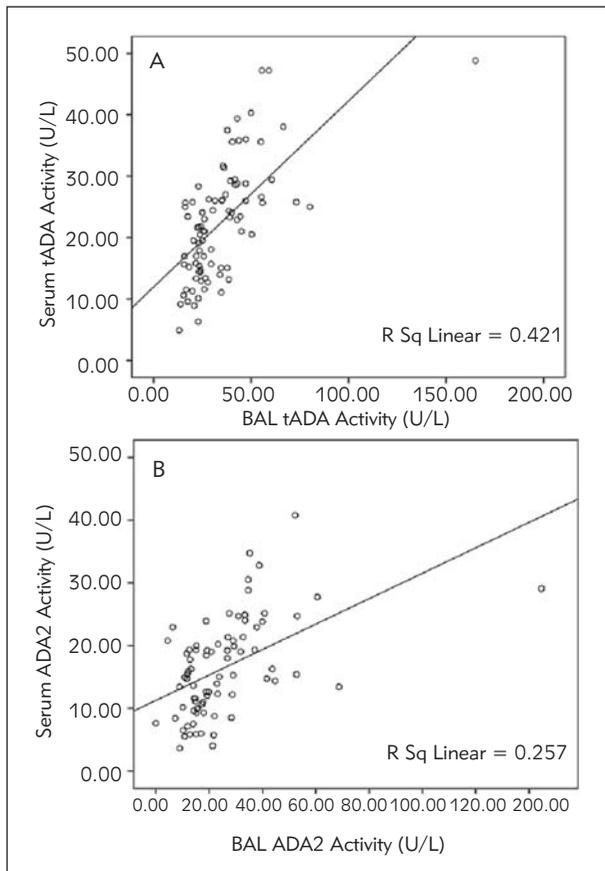


Figure 1 (A) Correlation of serum tADA activity with BAL tADA activity and (B) serum ADA2 isoenzyme activity with BAL ADA2 isoenzyme activity.

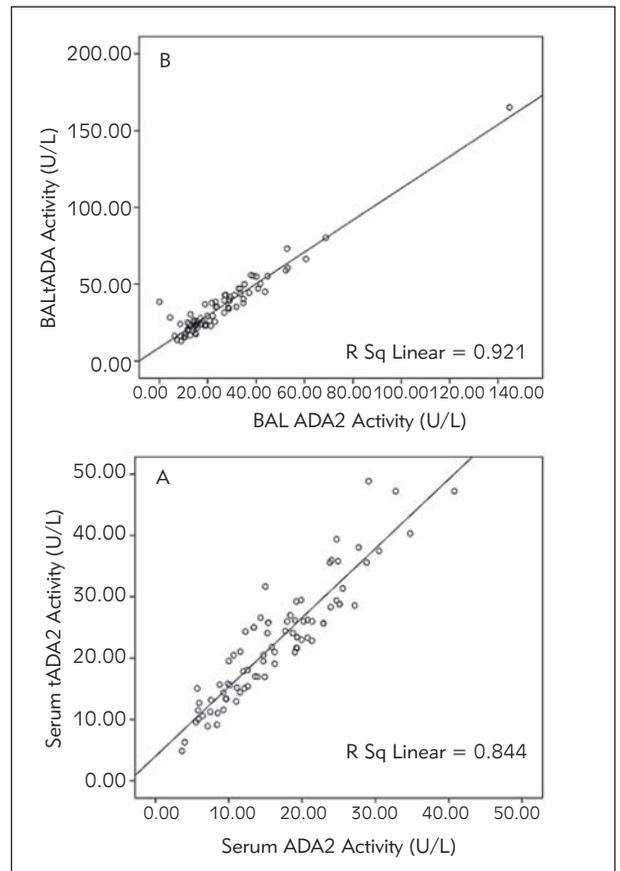


Figure 2 Correlation of serum (A) and (B) BAL total ADA and ADA2 isoenzyme activities in lung cancer and benign subjects.

Table II Sensitivity, specificity and accuracy, positive and negative LR and the PPV and NPV for serum and BAL adenosine deaminase activity.

		Cut-off point (U/L)	Sensitivity, %	Specificity, %	Accuracy, %	LR+	LR-	PPV	NPV
Serum	tADA	25	87	79	81	4.14	0.16	0.61	0.94
	ADA2	21.33	65	92	84	8.13	0.38	0.75	0.87
BAL	tADA	35.22	100	81	86	5.26	0.0	0.66	1
	ADA2	31.8	95	98	98	47.5	0.05	0.95	0.98

LR = Likelihood ratio; PPV = Positive predictive value; NPV = Negative predictive value.

ADA activity increased with the rising of ADA2 isoenzyme (Figure 2). Besides, BAL tADA activity did not correlate with BAL total protein levels, therefore demonstrating that it was not induced by the total protein levels. No correlation was detected between ADA activity and smoking (data not shown).

The cut-off values for sensitivity and specificity of serum and BAL ADA activity are given in Table II.

The ROC curve for ADA and its isoenzymes in serum and BAL is shown in Figure 3. Using a cut-off point of 35.22 U/L for BAL tADA, the corresponding sensitivity was 100% and specificity 81%. The positive and negative predictive values for BAL tADA were 0.66 and 1.0, respectively. Table II shows the positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio and negative likelihood ratio of tADA and its isoenzymes in BAL and serum of the

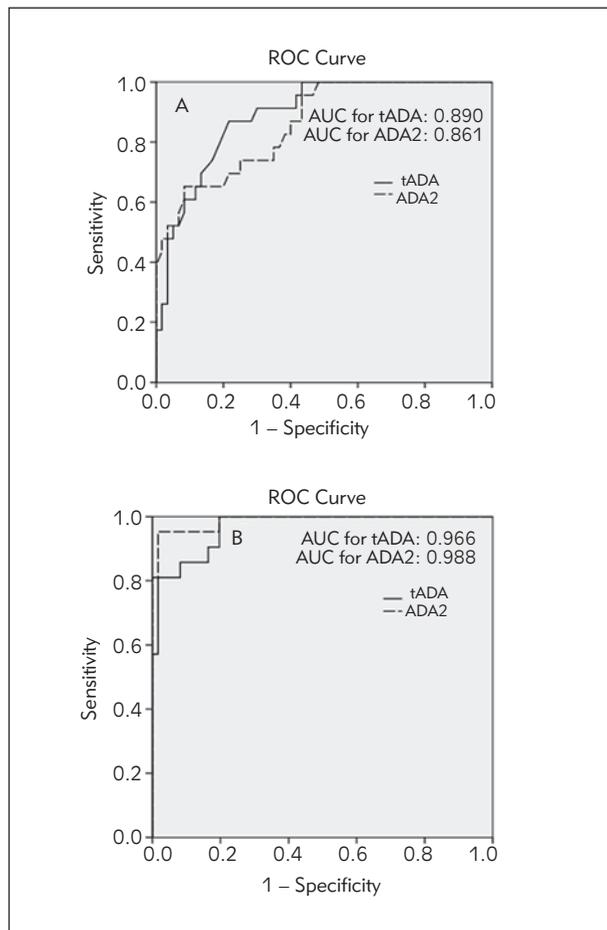


Figure 3 ROC curves for serum (A) and BAL (B) tADA and ADA2. ADA = Adenosine deaminase; AUC = area under the curve; ROC = receiver operating characteristic.

studied subjects. The highest (98%) specificity and highest (98%) accuracy could be obtained by detection of BAL ADA2. In addition, the highest (47.5) LR+ and the highest (0.95) PPV could also be obtained by detection of BAL ADA2. The highest (100%) sensitivity could be obtained by measurement of tADA activity in bronchoalveolar lavage fluid of the patients. Furthermore, the highest (1.0) NPV and the lowest (0.0) LR- could be obtained by detection of BAL tADA.

Discussion

To our knowledge, this is the first study in an Iranian population to investigate ADA activity in lung cancer. Several studies investigated the change of the adenosine deaminase activity in various cancerous patients. Many authors have shown the increase of serum tADA activity in these patients (4, 7, 23–26). Adapted to previous data, our results showed higher ADA activity in patients with lung cancer compared to controls.

Our results demonstrated that serum total ADA activity in lung cancer patients was significantly higher than in the negative pathologic tests group. This finding is supported by other reports (6), suggesting an increasing total ADA activity in lung cancer patients. On the other hand, our results neglected some studies that suggest no alteration in ADA activity in lung cancer patients (18). The simultaneous increase in total ADA and ADA2 isoenzyme activities, as observed in our study, has also been documented previously in other cancers such as breast and ovarian cancer (4, 7). Some studies suggest that the high tADA activity in cancerous patients has to do with its important role in the salvage pathway activity of cancerous tissue (27, 28). In addition, other researchers proposed that increased ADA activity may be due to a compensatory mechanism against the toxic effects of nucleotide metabolism substrates deposition in the tumour cells (29, 30).

The results of our study also confirmed significantly higher tADA and ADA2 isoenzyme activity in BAL fluid of cancerous patients. Additionally, we showed that BAL ADA activity has a positive correlation with the serum level of ADA. A similar direct correlation was also observed between ADA2 isoenzyme activity in BAL fluid and serum. This finding was imaginable, since a strong direct correlation of total ADA and ADA2 activities has been found in this study.

There are very few published clinical studies about the value of adenosine deaminase activity in breast cancer patients to date. In our present study, using a cut-off point of 38.22 U/L, the sensitivity and specificity of BAL fluid ADA were 100% and 81%, respectively. Ogata Y et al. (31) reported that by the measurement of tADA in pleural fluid and using a cut-off level of 36.0 U/L we can differentiate lung cancer from tuberculosis. The related sensitivity and specificity for this cut-off value were 85.5% and 85.6%, respectively. ADA activity in BAL fluid of tuberculosis patients was found to be higher than in lung cancer patients (32–34). Dimakou et al. (31) mentioned that when using a cut-off level of 5 U/L for sputum ADA2, sensitivity and specificity were respectively 81.5% and 63.2%. In that study, the corresponding values for total sputum ADA, using a cut-off level of 16 U/L, were 55.6% and 100%. With regard to these studies, this test has a high specificity and sensitivity to differentiate a malignancy from other benign lung disease.

Furthermore, we showed that because of high sensitivity, specificity and accuracy, measurement of ADA2 activity in bronchoalveolar lavage fluid is a very useful marker for diagnosis of lung cancer. The sensitivity and specificity for tADA and ADA2 in BAL fluid are the highest values that have been reported for this test to date.

In summary, we showed significantly higher activity of total ADA and ADA2 isoenzyme in cancer-

ous patients compared to negative pathological test controls. A direct correlation of ADA and/or ADA2 activity was also observed in this study between BAL fluid and serum. It can be concluded that, among the other tests, determination of total ADA activity (especially ADA2) in BAL fluid might be a simple, quick and low-priced diagnostic tool for screening and monitoring lung cancer. However, further studies with a larger sample size are recommended.

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Conflict of Interest Statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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