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Original Article

The Value of LDH Level of BAL Fluid in Differentiating Benign from Malignant Solitary Pulmonary Nodules

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Abstract

Background: Serum lactate dehydrogenase (LDH) concentration is an indicator for tissue injury. It may be secreted locally in many conditions. For the first time, this study was performed to investigate the value of LDH level in bronchoalveolar lavage fluid (BALF) in differentiation of benign from malignant single pulmonary nodules (SPNs) and to assess its relationship with serum LDH levels.

Methods: This study was a prospective case-control clinical study. It included 59 patients with a SPN and 21 non-smoker healthy adult volunteers as controls. They underwent bronchoscopy with BAL, Transbronchial needle aspiration (TBNA), and transbronchial biopsy (TBB). Both total serum and BAL LDH levels were measured.

Results: The range of the BAL LDH levels in the control group was 4.60 -26 mIU/ml, in patients with benign nodule was 6 – 83 mIU/ml, and in those with malignant nodule was 33 -147 mIU/ml. Overall, the mean BALF LDH level was significantly higher in patients with a malignant pulmonary nodule (85.92 ± 28.31) as compared with that of either patients with a benign nodule (19.08 ± 18.35) ($p<0.0001$) or control group (12.16 ± 6.18) ($p<0.0001$). No significant difference between the absolute value of BAL LDH level in patients with benign pulmonary nodule and the control subjects was found ($p=0.23$). There was no correlation between BALF LDH and serum LDH level in patients with SPNs ($p=0.595$).

Conclusion: BALF LDH levels are increased in patients with malignant SPN, but had no significant rise in benign solitary pulmonary nodules. This factor is useful in differentiating benign from malignant SPNs. A low BAL fluid LDH level in a patient with SPN who does not have a tissue diagnosis may be deemed acceptable for observation and follow up. This may save patients the need for operative procedures.

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Keywords ● Bronchoalveolar lavage ● LDH ● nodules ● malignancy ● bronchoscopy

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Introduction

Flexible bronchoscopes have been in regular use for many years to investigate patients with solitary pulmonary nodules (SPNs).[1] The most important first

plan is to determine the likelihood of the nodule being malignant and then to decide whether the lesion should be removed. The diagnostic yield of the standard combination of wash, brush, and transbronchial biopsy (TBB) in patients with SPN has a wide variability and depends on the location, size, character of the border, and the ability to perform all sampling methods.[2,3] It often requires the use of procedures with higher risk, such as percutaneous needle biopsy and thoracotomy.[4]

On the other hand, serum lactate dehydrogenase (LDH) concentration is an indicator for tissue injury, which may be increased in a variety of interstitial diseases.

To our knowledge, BALF LDH concentration has not been measured in cases with SPN. In the present series, we have tried to investigate the value of BALF LDH levels for differentiation between benign and malignant SPNs.

Patients and Methods

In our study, a SPN was defined as a single, well-circumscribed, spherical radiographic opacity that measured 1–4 cm in diameter and was surrounded completely by aerated lung with no calcium visible on a standard chest radiograph.[5,6] There were no associated pulmonary infiltrations, hilar enlargement, atelectasis, or pleural effusion. The patients with SPN who were detected on screening chest radiograph, were enrolled. The minimum size of the nodule was defined as one cm in diameter. The maximum size was defined as four cm. Patients were excluded if they suffered from known malignancy. In addition, the patients with hemoptysis, proven cardiovascular diseases, and abnormalities of liver function tests were excluded. The patients with history of cigarette smoking were also excluded because smoking may cause a possible inflammatory process and lung damage. Only patients who had a normal endobronchial anatomy were included in the study.

The study was approved by the research committee of Shiraz University of Medical Sciences. After obtaining written informed consents, all the patients underwent chest radiography, thoracic computed tomography (CT), and abdominal sonography prior to any procedure. Complete cell count, blood chemistry, sputum cytological and microbiological examinations were requested. Sera were drawn for total LDH level and albumin at the time of bronchoscopy, prior to lavage.

Bronchoscopy and bronchoalveolar lavage was performed in all eligible patients. BAL, transbronchial needle aspiration (TBNA), and TBB was performed using a flexible fiberoptic bronchoscope (Olympus BF1T, Tokyo, Japan). It was done before any bronchial brushings. After local anesthesia, the bronchoscope was wedged for lavage into a subsegmental involved bronchus determined by thoracic CT. Three 50-ml aliquots of sterile physiologic saline solution, warmed at 37°C, were infused. Fluid was immediately recovered by gentle suction after each aliquot was introduced. Samples of the lavage fluid were taken for fungal, mycobacterial, and other bacterial cultures. In addition, the lavage fluid was passed through two sheets of gauze to eliminate mucus and stored at 0°. One small aliquot of this fluid was utilized to count the total cell number, and another was spun in a cytometer at 400 rpm for 10 min. The cell pellet was washed once in Hanks´ balanced salt solution (without calcium and magnesium). May-Grünwall-Giemsa stains smear served to identify differential profiles after cytospin preparation by counting 600 cells. Total cell counts were determined with a hemocytometer. Macrophages, lymphocytes, and neutrophils were counted, and results were expressed as percentages. Unconcentrated supernatant was frozen at -20°C before LDH and albumin were measured.

Total LDH concentration was determined on the supernatant. The rate of reduction of nicotinamide adenine dinucleotide (NAD) to NADH in the presence of L-lactate was utilized to quantitate total LDH activity.[7] It was reflected by the rate of absorbance increase at 340 nm. All BAL LDH results were directly expressed in milli-international units (mIU) and were also given as mIU/mg albumin when albumin was used for standardization. Albumin concentration in serum was measured by immunonephelometry.[8] The technique of immunoradiometric assay was used to quantitate the albumin concentration in the BAL fluids.[9]

The peripheral nodule was approached first by brush and then by TBNA and TBB under fluoroscopic guidance. Thoracotomy was performed for those patients in whom a definite diagnosis could not be established by bronchoscopy (bronchial washing, brush, TBNA, TBB) or by sputum cytology, sputum microbiology, etc.

Bronchoscopy with BAL and subsequent measurement of LDH and albumin were performed for 21 non-smoker healthy adult volunteers who acted as our control group, using the same methods described above. Serum LDH and albumin concentrations were measured immediately prior to BAL.

All the results are presented as mean ± SD unless otherwise indicated. The Student's *t* test, Chi-square, and Kruskal- Wallis tests were used for analysis of data. The Spearman

rank test was performed to test the correlation between the patients, BAL LDH levels, and the serum LDH concentrations.

Results

Thirty five males and 24 females with an average age of 46.8 ±9.1 years (range 18–63 years) were examined. The age range of the cases in the control group was 29 - 43 years (mean ± SD = 33.5 ± 4.2). There was a significant difference in the mean age of the patients with malignant and benign nodules (P=0.03). With respect to age, the two study group of patients significantly differed from the control group (P<0.005). In 49 patients (83%) there were no symptoms ascribable to the SPN. In the remaining, 10 cases had cough (n=7) and chest pain (n=3).

The localization of the abnormality was the right middle lobe in three patients (5.1%), the left upper lobe in 11 (18.6%), the right upper lobe in 13 (22.1%), the left lower lobe in 17 (28.8%), and the right lower lobe in 15 (25.4%). Table 1 shows the final diagnosis in the patients with SPN.

Table 1: Final diagnosis in the patients with SPN.

Type of the findings	Number of the patients
Squamous cell carcinoma	12
Adenocarcinoma	21
Large cell carcinoma	2
Small cell carcinoma	7
Hamartoma	4
Tuberculosis	2
Sarcoidosis	2
Nonspecific	9
Total	59

In 17 (28.8%) patients the histology was benign, and in 42 (71.2%) the specimens were malignant. Diagnosis was obtained by sputum cytological and microbiological examinations in three patients (5%), by bronchoscopic evaluations in 36 patients (61%), and by thoracotomy in 20 (34%).

The total volume of fluid retrieved and the total number of cells recovered were not significantly different between the patients with SPN and the control subjects (P=0.61) (table 2). The percentages (mean ± SD) of macrophages, lymphocytes and granulocytes were significantly higher in patients than the control cases (p=0.0008, 0.03 and 0.01, respectively) (table 2).

For proportions of macrophages and lymphocytes, there were no significant differences between the patients with a benign nodule and those with a malignant lesion (P=0.34 and P= 0.72 respectively) (table 3). The proportion of granulocytes was significantly higher in patients with a benign lesion compared with cases with malignancy (P=0.01).

The mean BAL fluid and serum LDH levels (mean ± SD) of all patients and control subjects are shown in table 4 and figure 1. The highest BAL LDH levels in the control group was 26 mIU/ml (range = 4.60 -26), in patients with benign nodule was 83 mIU/ml (range = 6 - 83), and in those with malignant nodule was 147 mIU/ml (range = 33-147). Overall, BAL LDH level was significantly higher in patients with a malignant pulmonary nodule compared with that of either patients with a benign nodule (P<0.0001) or control group (P<0.0001). No significant difference between the BAL LDH level in patients with benign pulmonary nodule and the control subjects was found (P=0.12). In patients with malignant pulmonary nodule, no significant correlations between BAL fluid LDH levels and the proportions of macrophages (Rho=0.03, P=0.80) lymphocytes (Rho=0.11, P=0.44), and granulocytes were detected (Rho=0.21, P=0.16). There was no significant correlation between BAL fluid LDH levels and the size of the malignant nodules either (P=0.63).

Table 2: The findings of bronchoalveolar lavage in patients with SPNs and normal control subjects.

	Patients with SPN (n = 59)	Control subjects (n = 21)	P value
Recovered fluid (ml)	163.18 ± 14.57	165.42 ± 11.57	0.61
Total cells, x 10^6	86.44 ± 8.44	85.90 ± 7.56	0.74
Macrophages (%)	89.84 ± 2.45	92.10 ± 2.07	0.0008
Lymphocytes (%)	6.40 ± 2.65	5.15 ± 2.04	0.03
Neutrophils (%)	2.96 ± 1.17	2.29 ± 1.18	0.01

Table 3: The findings of bronchoalveolar lavage in patients with a benign and a malignant solitary pulmonary nodule.

	Patients with a benign nodule (n = 17)	Patients with a malignant nodule (n = 42)	P value
Recovered fluid (ml)	163.88 ± 14.83	162.90 ± 13.42	0.56
Total cells, × 10^6	84.41 ± 9.48	87.26 ± 7.97	0.30
Macrophages (%)	90.474 ± 2.30	89.59 ± 2.49	0.34
Lymphocytes (%)	6.65 ± 2.49	6.30 ± 2.18	0.72
Neutrophils (%)	2.32 ± 1.02	3.22 ± 1.30	0.01

Table 4: Mean serum and bronchoalveolar lavage lactate dehydrogenase (BAL LDH) levels (mIU/ml) in cases of control group and patients with benign and malignant pulmonary nodules.

	BAL LDH (mIU/ml)	Serum LDH (mIU/ml)
Controls (n = 21)	12.16 ± 6.18**	146.23 ± 25.93 ¥
Patients with SPN (n = 59)	66.66 ± 39.89	135.78 ± 57.32
Patients with a benign nodule (n = 17)	19.08 ± 18.35***	147.11 ± 64.83 ¥¥
Patients with a malignant nodule (n =42)	85.92 ± 28.31*	131.19 ± 54.15 ¥¥¥

* P < 0.0001 (between patients with malignant and benign nodules), ** P < 0.0001 (between patients with malignant nodules and controls), *** P = 0.11 (between patients with benign nodules and controls).
¥ P = 0.23 (between controls and patients with malignant nodules), ¥¥ P = 0.95 (between patients with benign nodules and controls), ¥¥¥ P = 0.33 (between patients with malignant and benign nodules).

Table 5: Albumin levels and LDH/albumin ratios of BAL and serum (mean ± SD) in control group and patients with benign and malignant pulmonary nodules.

	Serum		BAL fluid	
	Alb (µg/ml)	LDH/Alb (mIU/mg)	Alb (mg/ml)*	LDH/Alb (mIU/mg)
Controls	37.28 ± 3.84	3.95 ± 0.79*	22.01 ± 18.30	572.17 ± 326.06 ¥
Patients with a benign nodule	37.82 ± 3.87	3.88 ± 1.56***	28.38 ± 12.85	755.65 ± 741.60 ¥¥¥
Patients with a malignant nodule	37.76 ± 3.63	3.46 ± 1.34**	35.44 ± 17.75	2889.27 ± 1411 ¥, ¥¥

* P = 0.13 (between controls and patients with malignant nodules, ** P=0.31(between patients with malignant and benign nodules), *** P = 0.86 (between patients with benign nodules and controls).
¥ P <0.0001 (between controls and patients with malignant nodules), ¥¥ P<0.0001 (between patients with malignant and benign nodules), ¥¥¥ P =0.31 (between patients with benign nodules and controls)

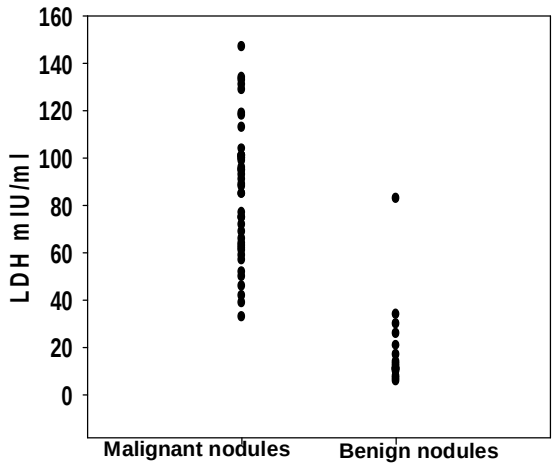

Figure 1: Bronchoalveolar lavage LDH levels (mIU/ml) in the patients with benign and malignant pulmonary nodules.

The serum LDH level was increased in one patient with a malignant nodule and two cases with a benign nodule (>250 mIU/ml). None of the cases of the control group showed an elevated serum LDH level. There was no significant correlation between BAL LDH levels and serum LDH levels (Rho=0.01, P= 0.94) in patients with a malignant nodule. The ratio of serum to BAL LDH level ranged from 0.53 to 3.65 (mean ± SD= 1.71 ± 0.87) in patients with malignant nodules, from 1.26 to 27.50 (mean ± SD = 11.25 ± 7.12) in patients with benign nodules and from 5.59 to 33.91 (mean ± SD = 14.93 ± 7.24) in control group. The difference between the two groups of patients was quite significant (P<0.0001), but no significant difference between the patients with benign nodules and the controls was found (P=0.12).

Table 5 shows allbumin levels and LDH/albumin ratios of BAL and serum in the patients and control subjects.

Discussion

Although most SPNs are benign,[10,11] primary malignancy may be found in approximately 35% of SPNs, and solitary metastases can account for another 23%.[12] The prevalence of malignancy in nodules varies widely, depending on the patient populations. Older age, a history of cigarette smoking, and a history of cancer increase the probability that an SPN is malignant.[13] Benign solitary pulmonary nodules are more common in the young and in nonsmokers.[14] Therefore, SPNs should be diagnosed, treated and managed in order not to overlook any treatable cancer.

Current usual methods to diagnose SPNs include sputum cytology, fine needle aspiration (FNA), bronchoscopy with brushings, washings and bronchial biopsy, and finally the most invasive technique of open lung biopsy.[3,15] Bronchoscopy may mostly be helpful in patients with central nodules. The mean costs of diagnostic tests and thoracotomy in our hospital are 600000 Rials (≈60 US Dollar) for bronchoscopy with BAL, 700000 Rials (≈70 US Dollar) for TBB, 850000 Rials (≈85 US Dollar) for CT-guided needle biopsy, and 1520000 Rials (≈152 US Dollar) for thoracotomy. The costs of diagnostic examinations include the costs of diagnostic procedure and radiological or/and pathological interpretations based on the estimates released by Iranian Ministry of Health and Medical Education.

The BAL fluid LDH level may increase in a variety of interstitial lung diseases. The increased level of this enzyme may reflect cytotoxicity.[16-18]. Pulmonary alveolar proteinosis and Pneumocystis carinii pneumonia are two well-documented causes of elevated serum LDH levels in the setting of diffuse lung disease.[19,20] The bronchoalveolar lavage fluid (BALF) LDH level may be increased in a variety of interstitial lung diseases.[21] The increased level of this enzyme may reflect cytotoxicity.[22] It is postulated that malignant lung lesion will cause cell damage. Therefore, elevated BALF LDH levels are expected to be seen secondary to tissue damage.[23,24]

According to this fact, the fundamental question in the present study is whether the determination of the BAL fluid LDH level can guide the physicians to improve their diagnosis, especially in complex cases. To the best of our knowledge, this is the first report regarding the measurement of LDH in the BALF of patients with SPN. The BALF LDH level was less than 32 mIU/ml in all cases with benign SPN, except in two patients whose SPNs were diagnosed as sarcoidosis (83 mIU/ml) and tuberculosis (34 mIU/ml). On the other hand, all patients with malignant SPN had an increased LDH levels (>33 mIU/ml) in their BALF as compared with the patients with benign SPN, except in one case whose malignant SPN was diagnosed as adenocarcinoma. The mean BALF LDH level was significantly higher in patients with malignant SPN than that of benign ones too.

The elevation of serum LDH levels in some patients with SPN may be due to other associated causes rather than lung injury. The presence of any hepatic disease and other organ abnormalities (as the causes of an increase in serum LDH level) were excluded in our patients. One out of 42 cases with a malignant SPN had an increased serum LDH level. The finding of serum to BAL LDH ratio was interesting. The values less than 3 were exclusively found in patients with malignant SPN, except in one case with sarcoidosis (benign SPN). The increased level of the lavage fluid albumin is also suggestive of an increased permeability of the alveolocapillary membrane.

The study shows that the lavage LDH/albumin ratio was significantly higher than the same ratio in serum in all patients with SPN and control subjects. Meanwhile, the serum/lavage LDH ratio was significantly higher in control subjects than the same ratio in patients with a malignant SPN ($P<0.0001$). We suppose that the rate of rise in the concentration of larger blood-born molecules in the BAL fluid may be slower than the smaller molecules, however, the BAL fluid LDH (molecular weight =140,000 daltons), was higher than that of its albumin concentration (molecular weight =69,000 daltons). Therefore, it may be postulated that the elevated BAL LDH level is mainly caused from its local production secondary to tissue damage, rather than reflecting passive leakage from the circulation to alveoli. If the passive transudation from blood to alveoli is considered as the sole cause of increased BAL LDH concentrations, the BAL LDH/albumin ratio should be higher than the same ratio in the serum.

No correlation was observed between BAL and serum LDH levels in cases with a malignant SPN. Meanwhile as it was indicated, the serum LDH level was not increased in none of the patients with a malignant SPN, except in one case whose nodule diagnosed as metastatic disease. Therefore, this study may suggest that the elevated total serum LDH in this case may be a reflection of lung tissue injury. In other words, increased backflow of the LDH molecule produced within the alveoli into the circulation through the alveolocapillary barrier in the presence of an inflammatory state may lead to the elevated serum LDH level in this case with metastatic disease.

Although the problem of measuring noncellular components in BAL has never been solved and the normal range for BAL LDH concentration has not been clearly established yet, a very low value (possibly less than 30 mIU/ml) may help to exclude the diagnosis of malignant nodule. In addition, a serum to BAL LDH ratio of more than 3.5 may highly reject the diagnosis of malignant nodule too. Further investigations are needed for a more clear elucidation of this issue.

In conclusion, determination of the BAL fluid LDH level in flexible bronchoscopy may be useful in the diagnosis of peripheral pulmonary nodules eluding diagnosis by other techniques. A low BAL fluid LDH level in a patient with SPN who does not have a tissue diagnosis may be deemed acceptable for observation and follow up. This may reduce the need for operative procedures. We encourage further studies addressing the improvement of this method in the evaluation of SPNs.

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