DIAGNOSTIC VALUES OF PLEURAL FLUID, AND SERUM C-REACTIVE PROTEIN, ADENOSINE DEAMINASE, AND LACTATE DEHYDROGENASE LEVELS AND PLEURAL FLUID/SERUM RATIOS IN THE DIFFERENTIATION OF MALIGNANT FROM BENIGN PLEURAL EFFUSIONS

MALİGN VE BENİGN PLEVRAL EFÜZYONLARIN AYRIMINDA PLEVRAL VE SERUM C-REAKTİF PROTEİN, ADENOZİN DEZAMİNAZ VE LAKTİK DEHİDROGENAZ DÜZEYLERİNİN TANISAL DEĞERİ

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SUMMARY

The aim o f the present study was to investigate the roles of serum and pleural fluid C-reactive protein (CRP), adenosine deaminase (ADA), and lactate dehydrogenase (LDH) levels in the differentiation of malignant from benign pleural effusions.

Totally, 103 patients with pleural effusions admitted to the hospital between March 2006 and June 2007 were enrolled in the study. Pleural fluid and serum samples were obtained from all cases upon admission to the hospital. Serum and pleural fluid CRP, ADA, and LDH levels and pleural fluid/serum CRP, ADA, and LDH ratios were compared between the groups with benign and malignant pleural effusions.

Cytologic assessment revealed that pleural fluids were benign and malignant in 75 and 28 cases, respectively. Of the cases with benign cytology, 29 were tuberculous pleurisy, 16 were non-specific

ÖZET

Plevral sıvıların malign-benign ayrımında serum ve plevral sıvı C-reaktif protein (CRP), adenozin deaminaz (ADA) ve laktik dehidrogenaz (LDH) düzeylerinin rolü araştırılmıştır.

Mart 2006 - Haziran 2007 döneminde hastanemize başvuran 103 plevral sıvılı olgu çalışmaya dahil edildi. Tüm olgulardan, hastaneye ilk başvuruları sırasında plevral sıvı ve serum örnekleri alındı. Benign-malign plevral sıvılı olgular arasında, serum ve plevral sıvı CRP, ADA, LDH düzeyleri ile plevral sıvı/serum CRP, ADA, LDH oranları karşılaştırıldı.

Sitolojik değerlendirmeye göre plevral sıvıların 75'i benign, 28'i malign sitolojide idi. Benign sitoloji olarak gruplanan olguların 29'u tüberküloz plörezi, 16'sı nonspesifik plörezi, 13'ü ampiyem-parapnömonik plörezi ve 17'si de KKY'ne bağlı plörezili idi. Plevral sıvı ve serum

pleurisy, 13 were empyema/parapneumonic pleurisy, and 17 were pleurisy associated with congestive heart failure (CHF). There were no contributions of pleural fluid and serum CRP and ADA levels, and pleural fluid/serum CRP, ADA, and LDH ratios in the differentiation of malignant from benign pleurisy. Only the serum LDH level was significantly higher based on the receiver operating characteristics (ROC) analysis in cases with malignant pleurisy (p=0.018). Logistic regression analysis also established that the serum LDH level was significantly higher in cases with malignant pleurisy (p=0.006).

It was concluded that serum LDH level can have a contribution in the differentiation of malignant from benign pleural effusions.

CRP, ADA düzeylerinin ve ayrıca plevral sıvı/serum CRP, ADA ve LDH oranlarının malign-benign plörezi ayrımında bir katkısı görülmedi. Sadece serum LDH düzeyi malign plörezi olgularında ROC analizi ile istatiksel açıdan anlamlı olarak yüksek idi (p:0.018). Ek olarak uygulanan lojistik regresyon analizinde de, serum LDH düzeyi malign plörezili olgularda istatistiki açıdan anlamlı olarak yüksek saptandı (p:0.006)

Malign-benign plevral sıvı ayrımında serum LDH düzeyinin katkısı olabileceği sonucuna varılmıştır.

INTRODUCTION

Rapid and accurate diagnosis remains the major clinical challenge in patients with suspected pleural infection. Since clinical data may be elusive, measurement of biomarkers in pleural fluids might provide a reliable tool for estimating of the aetiology (1).

In patients with pleural effusions, the first step in determining aetiology is to establish whether the fluid is a transudate or an exudate, for which Light's criteria offer the most efficient differentiation (2,3).

Lactate dehydrogenase (LDH), adenosine deaminase (ADA), and C-reactive protein (CRP) levels are higher in exudative pleural effusions when compared to transudative pleural effusions. Elevated pleural fluid ADA levels, observed in cases with tuberculous pleurisy, also tend to increase in cases with lymphoma, empyema, rheumatoid arthritis, and malignancy. Isoenzymes of LDH have been demonstrated to elevate in malignancies, such as mesothelioma, lymphoma and small cell lung carcinoma. It has also been reported that CRP levels are lower in patients with malignant pleural effusions when compared with pleural effusions caused by infections (4).

The aims of the present study were to measure the pleural fluid and serum concentrations of CRP, ADA, and LDH, as well as pleural fluid/serum ratios in patients with different causes of pleural effusions and to differentiating malignant from benign effusions.

MATERIALS AND METHODS

Consecutive patients admitted with pleural effusions to the Chest Diseases Outpatient Clinic of our hospital between March 2006 and June 2007, were enrolled in the present study. The local Ethics Committee approved this study, and all patients gave informed consent for the analysis of stored specimens for future research.

Patients were excluded if they had hemorrhagic diathesis, respiratory failure, history of therapy for the existent pleural effusion, inadequate amount of fluid for thoracentesis, inability to cooperate and terminal illness. Additionally patients who were on anticoagulant therapy, and who

declined to give consent to thoracentesis and pleural biopsy, were also excluded.

Following clinical and radiologic examinations, all patients enrolled in the study underwent thoracentesis, and venous blood samples were simultaneously collected. Pleural fluid samples of the patients were categorized as transudates or exudates according to Light's criteria. Albumin gradient was also used to differentiate transudative and exudative fluids in patients receiving diuretics. All patients underwent serum and pleural fluid LDH, ADA, and CRP measurements. Pleural fluid cytology was evaluated, acid-resistant bacilli (ARB), and non-specific culture analyses were performed with pleural biopsies, when needed, to establish the etiology. Based on the results of those studies, the subjects were categorized into 5 main groups: tuberculous pleurisy (group 1), malignant pleurisy (group 2), non-specific pleurisy (group 3), empyema-parapneumonic pleurisy (group 4), and pleurisy.

A pleural effusion was categorised as malignant if malignant cells were detected on cytological examination of the pleural fluid or biopsy specimen. Tuberculous pleuritis was diagnosed if Löwenstein cultures of pleural fluid, pleural biopsy tissue samples were positive or on the presence of granulomatous inflammation with caseous necrosis in pleural biopsy samples. Parapneumonic effusions/empyema referred to those associated with bacterial pneumonia. The patients were diagnosed with non-specific pleurisy if the patient underwent at least 3 pleural punctions and was established with benign cytology, and if the patient had no specific diagnosis following pleura biopsy, and in case of reduction or stabilization of the fluid at the 6-12 month follow-up visits. The diagnosis for pleural fluid associated with CHF was established if the fluid had a transudative character and was accompanied by clinical and radiologic heart failure findings.

Serum and pleural fluid LDH, ADA levels were measument daily with Olympus AU-640 autoanalyzer. Normal reference values were accepted for ADA activity 0-15 U/L in serum and 0-24 U/L in pleural fluid and for LDH activity 220-450 U/L for serum and pleural fluid.

CRP was established in serum and pleural fluid samples daily, by nephelometric Array-360 device (Beckman Coulter Inc., Fullerton, CA, USA) using C-reactive protein (CRPMPE) kits (Beckman Coulter Inc). The upper and lower limits for serum and pleural fluid CRP were 0.00-0.800 mg/L.

SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to carry out statistical analyses of the data obtained in the study. One-way ANOVA and Student's t-test were used for intergroup comparisons of parametric data. Post-hoc tests were used in the assessment of data established to be significant in one-way ANOVA test. The Mann-Whitney U and Kruskal-Wallis tests were used in intergroup comparisons of parametric data without normal distribution. Receiver operating characteristics (ROC) and logistic regression tests were used to investigate diagnostic values. The level of confidence was 95% and, the significance level was considered as $p<0.05$.

RESULTS

The present study was carried out in 103 patients (78 males (75.7%); and 25 females (24.3%); age range, 17-86 years) diagnosed with pleurisy. The mean age was 56.42± 18.37 years for males and 52.48±16.98 years for females.

The distribution of the 103 patients according to the groups was presented in Table 1.

Cytologic evaluation revealed the pleural fluid to be benign and malignant in 75 and 28 cases, respectively. The mean ADA, CRP, and LDH levels in serum and pleural fluid are presented in Table 2.

A comparison of the patients in terms of the presence of malignant and benign effusions revealed that only the serum LDH level was significantly higher in patients with malignant pleurisy (p=0.038). The distribution of the CRP, LDH and ADA values is presented in Table 3.

No significant difference was observed in terms of pleural fluid/serum CRP, LDH, and ADA levels, in the differentiation of malignant from benign pleurisy (p>0.05). The distribution of these values is presented in Table 4.

Furthermore, ROC analysis was carried out to evaluate the diagnostic value of pleural fluid/serum CRP, LDH, and ADA ratios in the differentiation of malignant and benign

Table 1. The mean pleural fluid and serum ADA, CRP, and LDH values in patients.

	Tuberculous pleurisy (n=29)	Malignant pleurisy (n=28)	Non-specific pleurisy (n=16)	Empyema-parapneumonic pleurisy (n=13)	Pleurisy associated with CHF (n=17)
CRP					
Pleural fluid	4.65±3.71	2.43±2.68	2.16±1.70	820±7.15	1.72±1.93
Serum	7.56±6.27	6.28±9.97	3.46±4.29	15.03±12.43	4.33±3.54
LDH					
Pleural fluid	1031.52±871.23	1606.57±1913	584.0±551.84	4129.15±5273.0	314.41±141.52
Serum	455.97±348.13	1072.9±1445.8	334.13±123.04	443.55±291.8	652.50±415.97
ADA					
Pleural fluid	46.76±20.25	24.18±32.21	16.77±9.67	118.98±225.51	10.90±5.14
Serum	45.83±25.74	37.12±47.72	31.25±22.98	54.38±42.53	53.84±57.41

CRP: C-reactive protein, LDH: Lactate dehydrogenase, ADA: Adenosine deaminase, CHF: Chronic heart failure

Table 2. The mean pleural fluid and serum CRP, LDH, and ADA levels in malignant and benign effusions.

	Malignant	Benign	p
CRP			
Pleural fluid	2.43±2.68	4.14±4.54	>0.05
Serum	6.28±9.97	7.25±7.88	>0.05
LDH			
Pleural fluid	1606.57±1913.20	1310.43±2574.97	>0.05
Serum	1072.96±1445.86	470.67±333.77	0.038
ADA			
Pleural fluid	24.18±32.21	45.19±100.40	>0.05
Serum	37.12±47.72	46.46±38.47	>0.05

CRP: C-reactive protein, LDH: Lactate dehydrogenase, ADA: Adenosine deaminase

Table 3. Pleural fluid/serum ratios of CRP, LDH, and ADA lin malignant and benign effusions.

	Benign	Malign	p
Pleural fluid/serum CRP	0.71±0.72	0.83±1.14	0.866
Pleural fluid/serum LDH	3.77±9.29	2.59±4.37	0.848
Pleural fluid/serum ADA	1.30±1.76	1.09±1.28	0.709

CRP: C-reactive protein, LDH: Lactate dehydrogenase, ADA: Adenosine deaminase

Table 4. ROC analysis for pleural fluid and serum CRP, LDH, and ADA levels.

				95% confidence interval	
	B	S.E.	p	Upper Limit	Lower Limit
CRP					
Pleural fluid	0.373	0.067	0.066	0.241	0.505
Serum	0.370	0.069	0.059	0.235	0.505
LDH					
Pleural fluid	0.611	0.064	0.106	0.485	0.738
Serum	0.663	0.066	0.018	0.534	0.792
ADA					
Pleural fluid	0.366	0.066	0.052	0.237	0.495
Serum	0.344	0.069	0.023	0.209	0.478

ROC: Receiver operating characteristic, CRP: C-reactive protein, LDH: Lactate dehydrogenase, ADA: Adenosine deaminase, S.E.: Standard error

Table 5. ADA and LDH values calculetad with ROC analysis in differentiating malignant from benign pleural fluids.

					95% confidence interval	
	B	S.E.	p	OR	Upper Limit	Lower Limit
LDH serum	0.001	0.000	0.006	1.001	1.000	1.002
ADA serum	-0.011	0.008	0.186	0.989	0.973	1.005
Constant	-1.247	0.458	0.006	0.287		

ROC: Receiver operating characteristic, LDH: Lactate dehydrogenase, ADA: Adenosine deaminase, S.E.: Standard error, OR: Odds ratio

effusions. The area under the curve (AUC) was not significant.

When the same analysis was carried out for pleural fluid and serum CRP, LDH, and ADA levels, the serum LDH levels were significantly higher (p=0.018) and the serum ADA levels were significantly lower (p=0.023) in malignant effusions. ROC analysis is presented in Table 5.

Since a high AUC value was obtained for serum LDH and ADA levels in ROC analysis, logistic regression analysis was carried out to establish the diagnostic value of these two parameters in differentiating malignant from benign pleurisy. It was shown that while serum LDH value was directly associated in differentiating malignant from benign pleurisy, the serum ADA value had

no significant association (p=0.006 and p=0.186, respectively; Table 6).

DISCUSSION

Although it is easy to establish the presence of a pleural effusion clinically and radiologically, it is not always easy to determine the etiology. Diagnosis may not be ascertained for some patients despite the performance of all diagnostic steps, such as imaging methods, cellular, micro-biologic, and biochemical analyses of the fluid, cytologic examination, and closed and open pleural biopsies (2,4). Additionally, cytological examination of suspected malignant pleural effusion can result in false-negative rates of up to 40% (5). Diagnostic difficulties have led to the search of novel markers for pleural effusions.

LDH elevation is an indicator of pleural inflammation. A significant elevation is observed in pleural fluid LDH levels and pleural fluid/serum LDH ratios in cases with parapneumonic, malignant and tuberculosis effusions (6). LDH has five isoenzymes. Certain studies have reported that while isoenzyme patterns are useful in differentiating transudates from exudates, they have no contribution in the classification of pleural fluids (7,8). In the present study, we also observed that LDH levels were higher in exudative effusions when compared to transudative effusions. Parapneumonic, malignant, and tuberculosis-related pleural effusions had higher LDH levels than those induced by other causes. However, no significant difference was observed between the pleural fluid/serum LDH ratios of tuberculous and parapneumonic effusion cases.

It is known that cells that reproduce rapidly in the tissues have higher ADA activity compared with the cells that do not proliferate. While pleural fluid ADA levels higher than 70 U/L is generally interpreted in favor of tuberculous pleurisy, values lower than 40 U/L suggest causes unrelated to tuberculosis. This enzyme can also elevate in cases of lymphoma, empyema, rheumatoid arthritis, or malignancy (9,10). In the present study, it was observed that ADA levels and pleural fluid/serum ADA ratios were significantly higher in exudative effusions. Moreover, pleural fluid ADA levels were established to be significantly higher in tuberculous and parapneumonic effusions (PPEs) when compared with other pleural effusions. However, pleural fluid/serum ADA ratios were not established to offer a contribution in the differentiation of malignant from benign cases.

CRP, an acute-phase reactant released from the liver, is a common diagnostic test within hospital laboratories for the screening or monitoring of infections and noninfectious inflammatory diseases. CRP levels have been studied in pleural fluid and have been found to be higher in PPEs than in other types of exudative or transudative effusions (11-13). Turay et al. (11) has established that pleural fluid CRP level is significantly lower in the transudative group, and higher in the subgroup with parapneumonic effusions in the exudative group. A study conducted in Spain has reported that pleural fluid CRP levels lower than 20 mg/L suggested malignant origins, while values over 45 mg/L eliminated the probability of malignancy. It was also claimed that pleural fluid CRP levels have as much sensitivity and specificity as tumor markers. However, the study also emphasized the fact that the underlying reasons have not been clearly elucidated (12). In the present study, it was noted that CRP level and pleural fluid/serum CRP ratio in the transudative group was lower

than the values in the exudative group, whereas CRP levels were significantly higher in the parapneumonic and tuberculous pleurisy groups in comparison with the other subgroups. No significant difference was observed between the CRP levels and pleural fluid/serum ratios of malignant and benign effusions. Thus, it is suggested that CRP levels in pleural fluid can play a role in the differentiation of exudative and transudative effusions, but cannot be of assistance in differentiating malignant from benign pleurisy.

Porcel et al's findings in a large series of patients provide data on the value and accuracy of some of biomarkers (CRP, sTREM and LBP) measured in the pleural fluid to diagnose infectious effusions. They showed that a pleural fluid CRP level >80 $mg.L^{-1}$ argues for the presence of a PPE (likelihood ratio positive (LR+) 7.4), whereas CRP levels <20 $mg.L^{-1}$ are a strong indicator against an infectious pleural effusion, whether of bacterial or mycobacterial nature (likelihood ratio negative (LR-) 0.22) (14).

A recent study has demonstrated that pleural fluid ADA and CRP levels can a useful combination in the differential diagnosis of pleural effusions of malignant, tuberculous, and parapneumonic nature. As a result of this study, CRP levels lower than 4 mg/dL accompanied by an ADA level over 45 U/L, and an ADA level lower than 40 U/L accompanied by a CRP level over 6 mg/dL, and a CRP level lower than 4 mg/dL are recommended to be accepted as a high probability of tuberculosis, parapneumonic pleurisy, and malignant pleural effusions, respectively (15). In the present study, although no cut-off values were set, the results demonstrated that patients with malignant pleurisy had lower pleural fluid CRP levels and higher LDH levels.

In the present study, ROC analysis was also conducted to establish the diagnostic significance of pleural fluid/serum CRP, LDH, and ADA ratios in malignant from benign pleurisy differentiation. However, no additional diagnostic contribution has been observed. When the same analysis was carried out for pleural fluid and serum CRP, LDH, and ADA levels, significant differences were established in terms of serum LDH and ADA levels to differentiate malignant from benign pleurisy. In the logistic regression analysis carried out to establish the individual significance of LDH and ADA levels in the differentiation of malignant from benign pleurisy, though ADA levels were found to have no contribution serum LDH was observed to be directly associated.

In conclusion, in the differential diagnosis of malignant from benign pleural fluids although no significant contribution of serum and pleural fluid ADA and CRP levels were observed, serum LDH levels accompanied by clinical findings were established to be beneficial in the differential diagnosis. However, we believe that further biochemical markers have to be developed to assist the differential diagnosis of malignant from benign pleural effusions.

REFERENCES

1. Hassan T, Al-Alawi M, Chotirmal SH, McElvaney NG. Pleural fluid analysis: standstill or a work in progress? Pulmonary Medicine 2012; doi:10.1155/2012/716235.
2. Light RW. Pleural effusion. N Eng J Mec 2002; 346(25): 1971-7.
3. Light RW, MacGregor MI, Luchsinger PC, Ball WC. Pleural effusions: the diagnostic separation

of transudates and exudates. Ann Intern Med 1972; 77: 507-13.

4. Light RW. Clinical manifestations and useful tests. In: Light RW (Ed). Pleural disease. Baltimore: Williams & Wilkins. 1995: 36-74.
5. Rakha EA, Patil S, Abdulla K, Abdulkader M, Chaudry Z, Soomro IN. The sensitivity of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. Diagnostic Cytopathology 2010; 38(12): 874-9.
6. Ernam D, Atalay F, Hasanoglu HC, Kaptan O. Role of biochemical tests in the diagnosis of exudative plevral effusions. Clinical Biochemistry 2005; 38: 19-23.
7. Lossos IS, Breuer R, Intrator O, Sonenblick M. Differential diagnosis of pleural effusion by lactate dehydrogenase isoenyzme analysis. Chest 1997;111: 648-51.
8. Cobben NA, van Belle AF, Pennings HJ, et al. Diagnostic value ol lactate dehydrogenase isoenzyme pattern in pleural effusions. Br J Chem Clin Biochem 1997: 35: 523-8.
9. Piras MA. Gakis C, Budroni M. Andreoni G. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. Br Med J 1978; 2: 1751-2.
10. Ak G, Alatas F, Metintas M, et al. Tüberkülozlu olguların genel özellikleri. Toraks Dergisi 2002; 3(1): 45-51.
11. Yılmaz Turay U, Yıldırım Z. Turkoz Y, et al. Use of pleural fluid C-reactive protein in diagnosis of pleural effusions. Respir Med 2000; 94: 432-5.
12. Garcı´a-Pachon E, Llorca I. Diagnostic value of C-reactive protein in exudative pleural effusions. Eur J Intern Med 2002; 13: 246-9.
13. Kiropoulos TS, Kostikas K, Oikonomidi S, et al. Acute phase markers for the differentiation of infectious and malignant pleural effusions. Respir Med 2007; 101: 910-8.
14. Porcel JM, Vives M, Cao G, Bielsa S, Ruiz-Gonza´lez A, Martı´nez-Iribarren A, Esquerda A. Biomarkers of infection for the differential diagnosis of pleural effusions Eur Respir J 2009; 34: 1383-9.
15. Daniil ZD, Zintzaras E, Kiropoulos T, et al. Discrimination of exudative pleural effusions based on multiple biological parameters. Eur Respir J 2007; 30: 957-64.

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