Distinctive Value of Malondialdehyde Between Exudate And Transudate Of Pleural Effusion in Patients with Pleurisy

PLÖREZİLİ HASTALARDAKİ PLEVRAL EFÜZYONLARIN TRANSÜDA-EKSÜDA AYIRIMINDA MALONDİALDEHİDİN TANI DEĞERİ

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Summary

Purpose: In this study, it was aimed to investigate the value of Malondialdehyde (MDA, one of the lipid peroxidation products) for distinction between transudates and exudates of pleural effusion in patients with pleurisy.

Materials and Methods: A total of 30 cases with pleural effusion (15 exudative and 15 transudative) were taken into the study. MDA levels in serum and pleural fluid for each case were measured by thiobarbituric acid (TBA) method. MDA ratios of all the patients were determined by dividing pleural fluid MDA to serum MDA levels.

Results: There was not any significant difference ($p>0.05$) between the mean serum MDA values of groups with transudate (6.2±0.48 nmol/ml) and exudates (7.1±1.32 nmol/ml). On the other hand, difference between the mean pleural fluid MDA values of group with transudates (3.25±0.63 nmol/ml) and exudates (5.81±1.25 nmol/ml) was found to be statistically significant ($p<0.001$).

Conclusion: We propose that transudate-exudate distinction can be made with a high sensitivity and specificity when the value of 6 nmol/ml for pleural fluid MDA level (100% sensitivity and specificity) and the ratio of 0.8 for pleural fluid/serum MDA ratio (95% sensitivity and 90% specificity) are used as cut off values. Proposed values and above fortify exudative pleural fluid.

Key Words: Malondialdehyde, Transudate, Exudate, Pleurisy

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Özet

Amaç: Bu çalışma, plörezili hastalarda, plevral efüzyonların transüda, eksüda ayırımında kullanılabilecek lipid peroksidasyon ürünlerinden biri olan malondialdehid (MDA)'in tanı değerinin araştırılması amacıyla yapıldı.

Materyal ve Metod: 15 eksüda ve 15 transüda özelliğinde plevral efüzyonlu hastanın oluşturduğu toplam 30 hasta çalışmaya alındı. Her hastanın plevra ve serum MDA düzeyleri "thiobarbituric acid" (TBA) metodu ile ölçüldü. Hastaların hepsinde MDA oranlan, plevral sıvı MDA düzeyinin serum düzeyine bölünmesiyle belirlendi.

Bulgular: Transüdalı grubun serum MDA düzeyleri (6.2±0.48 nmol/ml) ile eksüdalı grubunkiler (7.1±1.32 nmol/ml) arasında anlamlı fark yoktu ($p>0.05$). Diğer taraftan transüdalı grubun plevral sıvı MDA düzeyleri (3.25±0.63 nmol/ml) ile eksüdalı grubun plevral sıvı MDA değerleri (5.8l±1.25 nmol/ml) arasında istatistiksel olarak anlamlı fark bulundu (pO.OOl).

Sonuç: Bu sonuçlara göre, plevral sıvı MDA seviyesi olarak 6 nmol/ml değeri kriter olarak alındığında, plevral efüzyonların transüda-eksüda ayırımları yüksek spesifite (100%) ve sensitivite (100%) ile yapılabilecektir. Plevral sıvı / serum MDA oranı olarak 0.8 değeri kriter alındığında bu ayrım 95% sensitivite ve 90% spesifite ile sağlanabilecektir. Önerilen değerler ve üstü eksudatif plevral sıvıyı desteklemektedir.

Anahtar Kelimeler: Malondialdehid, Transüda, Eksüda, Plörezi

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Pleural effusions are divided into transudates and exudates. A transudative pleural effusion develops when the systemic factors influencing the formation or absorption of pleural fluid are altered so that pleural fluid accumulates. In contrast, an exudative pleural effusion develops when the pleural surfaces or the capillaries in the location where the fluid originates are altered such that fluid accumulates. Transudate is an ultrafiltration of the plasma. However, exudate usually results from inflammation or a pleural disease (1,2).

In a patient with pleurisy, first approach is to differentiate whether pleural liquid is transudate or exudate. This differentiation is an important step for etiologic diagnosis. For this purpose, several methods and criteria have been used. Nevertheless, since none of these differentiation criteria does not have 100% specificity and sensitivity, problems are encountered in diagnosis of some pleurisy cases (1-3). In cases who trustworthy results were not possible by conventional methods, determination of levels of MDA in pleural liquid is reported to contribute high sensitivity for differentiation (4,5).

Two sources could be suggested for pleural fluid MDA. The first source is plasma, and plasma proteins, which escape at an increased rate into the pleural space through the capillaries of inflamed pleura (6). The second source is local production through enhanced lipid peroxidation in inflammatory cells. Lipid peroxides are important products of the eicosanoid synthetic pathways responsible for inflammatory mediator production (7).

The lipid peroxidation starts with the removal of a hydrogen atom from the chain of a polyunsaturated fatty acid in the membrane structure due to the effect of a free radical formed invivo. Consequently the structure of the cell membrane changes and the protective part disappears. Therefore it is suggested that free oxygen radicals and lipid peroxidation have a role in the formation of the exudative pleural effusion and lipid peroxidation products may be used in distinction between transudate and exudate (8,9).

Although diagnostic value of MDA levels in pleural liquid has been known for a long time, there are limited numbers of studies reporting MDA as a criteria to differentiate transudate-exudate (3-5).

This study that is an original manuscript, was carried out to investigate their utilization for differential diagnosis between transudates and exudates by defining their sensitivity and specificity by determining serum-MDA levels and MDA levels in pleural fluids which might be transudates or exudates.

Materials and Methods

Samples utilized in this study were provided from Chest and Internal Diseases Services of Firat University College of Medicine and Elazig State Hospital. We retrospectively studied 30 patients diagnosed with pleural effusion based on clinical, radiological and laboratory findings. There were 5 female and 10 male of 15 patients exudate (For cases with exudate, mean age±SD: 49.42±11.01). Patients with transudates consisted of 2 female and 13 male (For cases with transudates, mean age±SD: 54.09±12.38). All the patients were consisted of the cases with pleural effusions including 15 transudates: 9 congestive heart failure, 6 liver cirrhosis and 15 exudates: 9 neoplasm, 3 tuberculosis and 3 Para pneumonia). The precise diagnosis of tuberculosis and neoplastic pleurisy were provided with pleural biopsy, cytologic examination of pleural fluid and/or ARB positive in phlegm (in cases with pulmonary tuberculosis) or pleural fluid. None of them was receiving any antioxidant or chemotherapeutic agent and had other pathology (out of pleurisy-causes) connected free radical increase; therefore, our study group was little as number.

Some of the well-characterized and accepted criteria were utilized based on separating the exudates from transudates (8,9). We accepted as exudate the cases having precise etiologic diagnosis of exudate-pleurisy and, in addition, pleural effusion having these criteria are as follows: Either a pleural fluid protein level above 3.0 g/dl or pleural fluid cholesterol level above 60 mg/dl and at least, one of Light's criteria consisted of three differentiating characteristics: 1) a fluid-to-serum protein ratio greater than 0.5; 2) pleural fluid LDH greater than 200 IU; and 3) a fluid-to-serum LDH ratio greater than 0.6. The cases not matching aforementioned biochemical parameters and histopathologic evidence in pleural biopsy for exudates, with the definite etiologic diagnosis causing development of transudate, were accepted to be transudate. As the sensitivity and specifity quantities for these criteria have been defined in

previous several studies as classical, almost, we did not examine them.

Pleural effusion and plasma samples simultaneously obtained from each case were stored at -20 °C and analyzed within 1 week of collection.

MDA levels were determined as an indicator of free radical activity and lipid peroxidation in biological samples by using thiobarbituric acid (TBA) method. It is the most commonly used and easily applied method which depends on MDA's reaction with thiobarbituric acid (10). In our study, this method (10) were also used and the absorbance was measured at 532 nm and the results were expressed as nmol/ml and thus, the lipid-peroxide products (MDA) were detected by the same thiobarbituric acid (TBA) method in the serum and in the pleural fluids. Biochemical processes and analysis were carried out and evaluated by Biochemistry Department, College of Veterinary Medicine.

During all the procedures the ethical standards for the local ethic comity of Firat University-College of Medicine in Turkey were obeyed. Results were expressed as mean±SD. Probabilities were calculated using Student's /-test (Paired t test) and SPSS package program. The limit of statistical significance was set at $p<0.05$. The ratio of sensitivity and specificity was calculated by the medical statistician of College of Medicine.

Results

Significant correlation was not found between the age, sex and smoking index in either group of patients ($p> 0.05$). Pleural fluid protein levels in all of the patients with transudate were below 3 g/dl. In 12 (80 %) of patients with exudate, this level was above 3 g/dl. LDH levels in 14 (93.3 %) patients with transudate were determined as below 200 IU and also in 12 (80 %) patients with exudate were defined to be above this level. Pleural fluid/serum ratios of LDH were found to be smaller than 0.6 in 13 (86.6 %) patients with transudate and higher than 0.6 in 10 (66.6 %) of patients with exudate. The pleural fluid/serum protein ratios were found to be below 0.5 in 11 (73.3 %) patients with transudate and above 0.5 in 13 (86.6%) patients with exudate. The pleural fluid cholesterol levels were below 60 mg/dl in 14 (93.3%) cases with transudate and above 60 mg/dl in 11 (73.3 %) cases with exudate. (Table 1).

When MDA levels were compared, it was found not any statistically significant difference ($p>0.05$) between the serum MDA levels of patients with transudate (mean MDA level in serum: 6.2±0.48) and exudate (mean MDA level in serum: 7.1±1.32). On the other hand, it was defined a highly significant difference ($p<0.001$) between their pleural fluid MDA levels (pleural fluid mean MDA level of patient with transudate: 3.25±0.63; pleural fluid mean MDA level of patient with exudate: 5.81+1.25) (Table 2).

We propose that transudate-exudate distinction can be made when a cut-off value is taken as 6 nmol/ml for pleural fluid MDA level (100% sensitivity and specificity) and the ratio of 0.8 for pleural fluid/serum MDA ratio are 95% sensitivity and 90% specificity.

Table 1. Criteria used in distinction of transudates and exudates

Pleural Fluid (n)	Protein n %	LDH n %	P/S LDH n %	P/S Prot n %	Cholesterol %
Transudate (15)	<3/g/dl 15(100%)	<200 U/L 14(93.3%)	<0.6 13(86.6%)	<0.5 11(73.3%)	<60 mg/dl 14(93.3%)
Exudate (15)	>3/g/dl 12(%80)	>200 U/L 12 (80%)	>0.6 10(66.6%)	>0.5 13 (86.6%)	>60 mg/dl 11 (73.3%)

LDH: Lactate dehydrogenase, P/S: Pleura/Serum, Prot: Protein

Table 2. Sensitivities and specificities connected cut-off values of MDA levels and MDA levels of patients with transudate and exudate

	MDA in the transudates n: 15	MDA in the exudates, n: 15	p value	Sensitivity	Specificity
Serum	6.2±0.48	7.1Ü.32	p> 0.05		
Pleural fluid	3.25±0.63	5.81Ü.25	p< 0.001		
Cut-off value (Pleural fluid)	<6 nmol/ml	>6 nmol/ml		100%	100%
Cut-off ratio (PleuVSerum)	<0.8	>0.8		95%	90%

MDA: Malondialdehyde. Pleu* : Pleural fluid. MDA Unit: nmol/m!

Discussion

When it was assessed a patient with a pleural effusion, the first question is to answer whether effusion is a transudate or an exudate. For many years, a pleural fluid protein level of 3.0 g/dl was used to separate transudates from exudates, with exudative pleural effusions characterized by a protein level above 3.0 g/dl. Use of this simple test leads to the misclassification of approximately 10% of pleural effusions (2). In the present study, the pleural fluid protein levels were found to be above 3 g/dl in 80.9% of the patients with exudate and below 3 g/dl in 100% of the patients with transudate. Light and his co-workers reported that pleural protein levels were below 3 g/dl in 84% of patients with transudate due to the congestive heart failure (1).

In recent years, some new tests were proposed to differentiate transudates and exudates. Valdes and co-workers reported that transudates tend to have a pleural fluid cholesterol level below 60 mg/dl, whereas most exudative pleural effusions have cholesterol levels that exceed this value (3). Another report demonstrated that transudates had a gradient between the serum and the pleural fluid for albumin that exceeded 1.2 g/dl while exudates had a gradient that was fewer than 1.2 g/dl (11). If it is thought that the patient has a transudative pleural effusion clinically, but Light's exudative criteria are met, then it is reasonable to measure the serum-pleural fluid albumin gradient. If this is above 1.2 g/dl, then the patient in all probability has a transudative pleural effusion. Use of the serum-pleural fluid albumin gradient alone will result in the misclassification of many exudates as transudates (2).

To date, when Light's criteria was compared to the proposed new tests, it was concluded that Light's criteria was still the best to separate exudates and transudates. It has been reported that exudative pleural effusions meet at least one of the criteria reported as Light's criteria whereas transudative pleural effusions meet none (2,11).

Romero et al. also suggested that Light's criteria were superior to the cholesterol in making the distinction. In the present study, Light's criteria were also found to be superior to the serum-effusion albumin gradient and the effusion cholesterol concentration (4).

In our study, when they were considered according to Light's criteria, the pleural fluid LDH levels in 93.9% of the patients with transudate were determined below 200 IU and the pleural fluid LDH levels in 80% of patients with exudate were above this level. The pleural fluid/serum ratios of LDH were determined to be smaller than 0.6 in 86.6% of the transudates and bigger than 0.6 in 66.6% of the exudates; the pleural fluid/serum protein ratios were found to be below 0.5 in ***13.3%*** of the transudates and above 0.5 in 86.6% of the exudates. In addition, the pleural fluid cholesterol level was below 60 mg/dl in 93.3% of the transudates and above 60 mg/dl in 73.3% of the exudates.

It has been suggested that lipid peroxidation products can be utilised in the transudate/exudate distinction (8,9). MDA levels of acute phase difference was reported to be considerable in rats in

which pleurisy was developed (12). Hammouda and co-workers reported that MDA levels of pleural fluid were markedly higher in exudates than in transudates ($p<0.001$) (13). When 4.5 μmol/L considered as cutt-off value for pleural liquid MDA, 100% accuracy is reported for sample differentiation. In addition, they reported that no sharply dividing line was evident for pleural fluid to plasma MDA concentration ratios (13).

In our study, we found that there was not any statistically significant difference ($p>0.05$) between the serum MDA levels of patients with transudate and exudate whereas there was a statistically significant difference ($p<0.001$) between the pleural fluid MDA levels of transudates and exudates. In conclusion, we propose that transudate-exudate distinction can be made with a better sensitivity and specificity than another several methods when the value of 6 nmol/ml for pleural fluid MDA level (100% sensitivity and specificity) and the ratio of 0.8 for pleural fluid/serum MDA ratio (95% sensitivity and 90% specificity) are taken as the cut-off values. We assert that the parameters obtained by this method may be the new criteria for diagnosis of exudative and transudative pleural fluid as significant.

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