HDL/LDL ratio: a useful parameter for separation of pleural transudates from exudates

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ÖZET

HDL/LDL oranı: Transüda-eksüda ayrımında kullanışlı bir parametre

Plevral efüzyonların tanısını koymada ilk basamak transüda-eksüda ayrımının yapılmasıdır. Çalışmamızda, plevral sıvıda hesaplanan HDL/LDL oranının transüda-eksüda ayrımındaki yerini araştırmayı amaçladık. Yirmisekizi transüda, 93'ü eksüda olmak üzere 121 plevral sıvı analiz edildi. Plevra sıvılarında kolesterol, HDL ve LDL düzeyleri ölçüldü. HDL/LDL oranı hesaplandı. HDL/LDL oranı transüdalarda eksüdalara oranla anlamlı ölçüde yüksek bulundu (p= 0.001). "Receiver Operating Characteristic (ROC)" eğrisi ile "cut-off" değer bulundu. HDL/LDL oranının maksimum sensitivite ve spesifisite ile transüda tanısı koyduran değeri saptandı. Transüdaları eksüdalardan ayıran HDL/LDL oranı 0.6 olarak belirlendi (sensitivite: %89, spesifisite %79). Plevral sıvılarda HDL ve LDL ölçümü ile HDL/LDL oranı hesaplanması, serum örneği almaya gerek kalmadan transüda-eksüda ayrımının yapılmasında uygun bir yöntemdir.

Anahtar Kelimeler: *Lipoprotein, HDL, LDL, plevral sıvı.*

SUMMARY

HDL/LDL ratio: a useful parameter for separation of pleural transudates from exudates

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The first diagnostic step in pleural effusions is the separation of transudates from exudates. We aimed in present study to investigate the value of HDL/LDL ratio for distinguishing between pleural exudates and transudates. Pleural fluids (PF) from 121 patients, including 28 transudates and 93 exudates were analyzed. The levels of cholesterol, HDL cholesterol and LDL cholesterol in PF were measured. The HDL/LDL ratio was calculated. HDL/LDL ratio found significantly higher in transudates than exudates (p= 0.001). Receiver operating characteristic (ROC) curves were generated and the cut off points determined to the highest level of accuracy and precision. The HDL/LDL ratio was to maximize sensitivity over specificity in

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the diagnosis of a transudative effusion. The usefulness of HDL/LDL ratio for identifying transudates was evaluated in terms of sensitivity and specificity. The value of pleural HDL/LDL ratio that best differentiated between transudates and exudates was 0.6 (sensitivity 89%, and specificity of 79%). Measurement of HDL and LDL in PF and calculating of HDL/LDL ratio can be proposed to aid for differentiation between pleural exudates and transudates with advantage of not requiring serum levels.

Key Words: *Lipoproteins, HDL, LDL, pleural fluid.*

The presence of pleural effusion in the pleural space is a common diagnostic problem in clinical routine. The first diagnostic step in pleural effusions is the separation of transudates from exudates. The criteria of Light et al have been used for the differentiation of transudative and exudative pleural effusions for the past 3 decades. According to these criteria; an exudate is characterized by a pleural fluid (PF) protein- to-serum protein ratio greater than 0.5, a PF lactate dehydrogenase (LDH) - to- serum LDH ratio greater than 0.6 or a PF LDH level greater than 200 IU (1). Since these criteria originally were proposed, other tests for the separation of transudates and exudates have been proposed. One proposed test is the level of total cholesterol in the PF (2-4). The first article in this subject written by Hamm et al., showed the diagnostic usefulness of the cholesterol content in PF (2).

Cholesterol is not found free in blood but, rather, is linked to lipoproteins. The preponderance of serum cholesterol (90%) is carried in the low-density lipoproteins (LDL) (52% of LDL weight is due to cholesterol) and high-density lipoproteins (HDL) (19% of HDL weight is due to cholesterol). It is supposed that PF cholesterol also is related to LDL and HDL (5).

In Pfalzer's study, all transudates and exudates were correctly classified by using a dividing line of 55 mg/dL for cholesterol and it suggested that the low cholesterol content in transudates is the result of the constantly low levels of LDL particles in these effusions. An other words, usefulness of cholesterol measurement in effusions is based on the lack of LDL in transudates and the higher appearance in exudates (6).

We aimed in present study to measure the value of PF HDL and LDL levels and investigate the value of HDL/LDL ratio for distinguishing between pleural exudates and transudates. It was hypothesized that the increase in capillary permeability and the reduced lymphatic removal leaded to the accumulation of large particles, such as LDL and PF HDL/LDL ratio would tend to be lower if the permeability of the pleura was higher.

MATERIALS and METHODS

A prospective study of 129 consecutive patients admitted for pleural effusion was carried out. All patients gave informed consent for participation in the study, which was approved by the Ethics Committee of the Gazi University. PF samples were obtained by thoracentesis from patients admitted to our department. Eight cases were excluded because either no cause was definitely found, or more than one cause was present. The remaining 121 cases composed the study group, and they were classified on the basis of clinical and laboratory diagnosis. The patients who had pulmonary embolism were classified on the basis of only Light's criteria.

The diagnosis of the disease causing the effusion was considered to be confirmed when the following conditions were met:

1. Congestive heart failure: Presence of an enlarged heart with clinical or echocardiographic evidence of cardiac dysfunction, and one or more of following alterations: elevated venous pressure, peripheral edema, tachycardia, or ventricular gallop, response to optimal medical treatment. Patients suspected of having respiratory infections, pulmonary emboli, or persistence of the effusion after adequate treatment of the cardiac insufficiency, were excluded,

2. Nephrotic syndrome: Presence of proteinuria greater than 3.5 g/day, edema and hypoalbuminaemia (less than 3.5 mg/dL),

3. Pleural malignancy: Cytological or histological demonstration of pleural involvement,

4. Tuberculosis: Presence of tuberculosis granulomas in pleural biopsy specimen or positive smear or culture of acid-fast bacilli,

5. Parapneumonic effusion: Clinically and radiologically confirmed pneumonia with no direct or indirect evidence of bacterial invasion of the effusion,

6. Pulmonary embolism: Presence high probability ventilation-perfusion scan and strong clinical suspicion and absence of malignancy, respiratory infection or heart failure.

PFs from 121 patients, including 28 transudates and 93 exudates were analyzed. Venous blood samples were obtained after an overnight fasting on the same day as thoracentesis. After clotting at room temperature, the samples were centrifuged for 10 minutes at 3000 g and stored at -70°C. Effusion samples were prepared in the same way, only material from the first thoracentesis was used. For each patient, the levels of cholesterol, HDL cholesterol and LDL cholesterol in PF were measured. The HDL/LDL ratio was calculated.

Total cholesterol levels were measured at Abbott Aeroset autoanalyzer using original reagents which is based on the formulation of Allain et al and modification of Roeschlau. In this method, cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide combines with hydroxybenzoic acid and 4-aminoantipyrine to form a chromophore which is quantitated at 500 nm (7,8).

HDL cholesterol levels were measured at Abbott Aeroset autoanalyzer using Scil Diagnostic reagents which is based on the enzymatic assay (9).

LDL cholesterol levels were calculated from the following formula by Friedewald et al.

LDL= Total cholesterol - [HDL + (Triglyceride/5)] (10).

Statistical Analysis

Means and standard deviations were calculated by conventional statistical methods. The comparison of the means was determined from the Mann-Whitney U test. Receiver operating characteristic (ROC) curves were generated and the cut off points determined to the highest level of accuracy and precision. The sensitivities and specificities were determined from the ROC curves. The 95% confidence intervals, positive and negative predictive values were calculated using standard methods. The data were analyzed via computer software (SPSS, version 11.0). $p < 0.05$ was considered to be statistically significant.

RESULTS

The 121 patients studied consisted of 39 women and 82 men with a mean age of 52.4 ± 19.8 years (range 16-98 years). Among the 28 (23.1%) patients with transudates, 9 (32.1%) were women and 19 (67.9%) were men, with a mean age of 63.9 ± 16.6 years (range 25-98 years). Among the 93 (76.9%) patients with exudates, 30 (32.3%) were women and 63 (67.7%) were men, with a mean age of 48.9 ± 19.4 years (range 16-84 years). The causes of the effusions are listed in Table 1. The mean HDL level was 21.37 ± 11.87 in exudates, 17.79 ± 7.64 in transudates (p= 0.17). The mean LDL level was 58.69 ± 29.8 in exudates and 12.32 ± 13.08 in transudates (p= 0.001). For each patient the HDL/LDL ratio was calculated and found

Table 1. The causes of the effusions.

	Frequency (n)	Percent (%)
Transudates		
Nephrotic syndrome	3	10.7
Congestive heart failure	24	85.7
Pulmonary embolism	1	3.6
Total	28	
Exudates		
Tuberculosis	38	40.8
Parapneumonic	18	19.4
Pleural malignancy	33	35.5
Pulmonary embolism	4	4.3
Total	93	

significantly higher in transudates than exudates (p= 0.001). The mean HDL/LDL ratio was 2.87 ± 1.98 in transudates and 0.46 ± 0.37 in exudates. The HDL/LDL ratio was to maximize sensitivity over specificity in the diagnosis of a transudative effusion, different cut off levels for HDL/LDL ratio were applied as shown Table 2 and Figure 1. The value of pleural HDL/LDL ratio that best differentiated between transudates and exudates was 0.59 (sensitivity 89%, and specificity of 80%).

The usefulness of HDL/LDL ratio for identifying transudates was evaluated in terms of sensitivity and specificity (Table 3). PF HDL/LDL ratio (0.6) measurement was equally good at differentiating between exudates and transudates. PF HDL/LDL ratio misclassified 22 of the 121, consisting of 19 exudates and 3 transudates with a sensitivity of 89%, a specificity of 79%, and an accuracy of 82%.

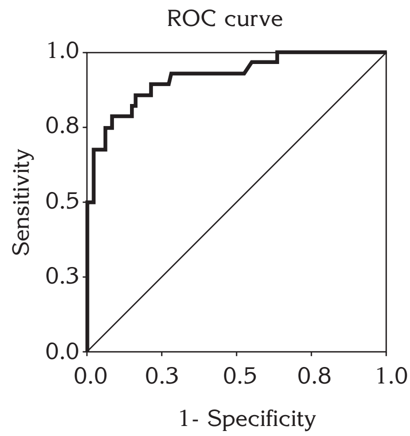

Figure 1. The receiver operator curves (ROC) for HDL/LDL ratio for separation between transudates and exudates.

DISCUSSION

The known lipoproteins are chylomicron, very low-density lipoproteins (VLDL), LDL, HDL and intermediate-density lipoprotein. Approximately 90% of the cholesterol in the blood is associated with LDL and HDL. LDL is synthesized in the vessels while HDL is synthesized in both the liver and the vessels. These lipids have an important role in cellular metabolism. But, the importance of the lipids in pleural physiology and in pleural disease remains to be determined (5).

Table 2. The sensitivity and specificity of the cut-off values of HDL/LDL ratio.

Positive if greater or equal than to	Sensitivity	1-Specificity
0.5	0.89	0.25
0.51	0.89	0.24
0.53	0.89	0.23
0.55	0.89	0.22
0.56	0.89	0.21
0.59	**0.89**	**0.20**
0.63	0.85	0.20
0.64	0.85	0.19
0.68	0.85	0.18
0.73	0.85	0.17
0.74	0.85	0.16

Table 3. Statistical analysis of the parameters used to classify transudates and exudates, expressed as percentage (95% confidence intervals).

	PF HDL/LDL ratio
Cut off	0.6
Sensitivity	89%
Specificity	79%
Accuracy	82%

Cut off value was obtained from ROC analysis. Sensitivity is TP/(TP + FN), specificity is TN/(TN + FP), accuracy is (TP + TN)/(TP + TN + FP + FN), where TP is the number of true positive diagnoses (number of transudates correctly diagnosed), TN is the number of true negative diagnoses (number of exudates correctly diagnosed), FP is the number of false positive diagnoses (number of exudates undiagnosed), and FN is the number of false negative diagnoses (the number of transudates undiagnosed).

For distinguishing between pleural transudates and exudates the most analyzed parameters have been the PF cholesterol level and the PF to serum cholesterol ratio. Hamm et al reported that cholesterol level was superior to Light's criteria in distinguishing transudates from exudates (2). The application of a cut off of 60 mg/dL yielded a sensitivity and specificity of 90 and

100%, respectively. Valdes et al, later confirmed this results, adopting a cut off point of 55 mg/dL (sensitivity 91%, specificity 100%) (11). In the other series, different cut off values were used (12-14). Gazquez et al, in spite of others authors, found pleural cholesterol to be a poorer method than the criteria of Light et al. Thus, applying the best cut off level (50 mg/dL), a sensitivity, specificity and accuracy of 84% was found (3). Pleural cholesterol is not standardized and the best cut off level for cholesterol in PF should be attained for each particular laboratory before introducing it in routine work-up.

It was recognized as early as at 1961 by Kellogg and Mann that, LDL level in exudates depends on pore size (15). Reports on lipid and lipoprotein analyses in PF were remained rare by this time. Cholesterol measurement has been used early in the diagnosis of chyliform pleural effusions. Lipoprotein electrophoresis and triglyceride determination have been suggested for the differentiation of chylothorax from nonchylous effusions (16).

In present study, we found that the mean LDL level was significantly lower in transudates than exudates. The mean level of HDL was also lower in transudates than exudates, but this difference was not significant. Therefore, the mean HDL/LDL ratio was found significantly higher in transudates than exudates. This can be explained by increased pleural permeability in exudates, which allows transporting of large molecules like LDL. Nevertheless, there was no significant difference between transudates and exudates in relation with HDL, which was also large molecule. So, there may be some other mechanisms affecting lipoprotein levels in PF.

When we reviewed other studies investigating these mechanisms, Vaz et al recently demonstrated that cholesterol levels in PF were related to serum cholesterol levels and to the permeability of the pleura. They found that the percentage of cholesterol associated with LDL and HDL in the PF was much lower than that associated with LDL and HDL in serum, suggesting that lipoproteins are modified once they enter the pleural space (5).

Pfalzer et al investigated the pathophysiologic mechanisms of cholesterol in pleural effusions and the possible diagnostic differences between inflammatory and malignant exudates. In conclusion of their study they suggested that the HDL was modified in PF (6).

Rerabek reported high LDL levels in 20 effusions caused by bacterial pleurisy and discussed the dependence of LDL level on the pore size of the inflamed pleural membrane. They concluded that the cholesterol content in pleural exudates simply reflect serum levels (17).

Raymond have described alterations of LDL level in interstitial inflammatory fluid that lead to enhanced degradation by macrophages. They demonstrated that leukopenia suppresses the modification of LDL and concluded that leukocytes were essential for modification (18). According to this suggest, the inflamed pleural space is a condition where leukocytes and protease are strongly activated and LDL level is higher.

In summary, the diagnostic usefulness of cholesterol measurement in effusions is based on the lack of LDL in transudates and the higher appearance in exudates. The low cholesterol content in transudates is the result of the constantly low levels of LDL particles in these effusions. Finally, we showed that the mean HDL/LDL ratio was significantly higher in transudates than exudates. The value of pleural HDL/LDL ratio that best differentiated between transudates and exudates was 0.6 (sensitivity 89%, and specificity of 79%). In other words, measurement of HDL and LDL in PF and calculating of HDL/LDL ratio can be proposed to aid PF differentiation with advantage of not requiring serum levels. For evaluating the mechanism of lipoproteins in pleural effusions, the further studies are needed.

REFERENCES

1. *Light RW, MacGregor MI, Luchsinger PC, Ball WC. Pleural effusions: the diagnostic separation of transudates and exudates. Ann Intern Med 1972; 77: 507-13.*
2. *Hamm H, Brohan U, Bohmer R, et al. Cholesterol in pleural effusions: a diagnostic aid. Chest 1987; 92: 296-302.*
3. *Gazquez I, Porcel JM, Vives M, et al. Comparative analysis of Light's criteria and other biochemical parameters*

for distinguishing transudates from exudates. Respir Med 1998; 92: 762-5.

4. *Metintas M, Alatas O, Alatas F, et al. Comparative analysis of biochemical parameters for differentiation of pleural exudates from transudates Light's criteria, cholesterol, bilirubin, albumin gradient, alkaline phosphatase, creatine kinase, and uric aid. Clin Chim Acta 1997; 264: 149-62.*
5. *Vaz MA, Teixeira LR, Vargas FS, et al. Relationship between pleural fluid and serum cholesterol levels. Chest 2001; 119: 204-10.*
6. *Pfalzer B, Hamm H, Beisiegel U, Ostendorf P. Lipoproteins and apolipoproteins in human pleural effusions. J Lab Clin Med 1992; 120: 483-93.*
7. *Allain CC, Poon LS, Chan CS, et al. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470-5.*
8. *Roeschlau P, Bernt E, Gruber WA. Enzymatic determination of total cholesterol in serum. Z Klin Chem Klin Biochem 1974; 12: 226-7.*
9. *Rifai N, Warnick GR, McNamara JR, et al. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin Chem 1992; 38: 150-60.*
10. *Li KM, Wilcken DE, Dudman NP. Effect of serum lipoprotein (a) on estimation of low-density lipoprotein cholesterol by the Friedewald formula. Clin Chem 1994; 40: 571-3.*
11. *Valdes L, Pose A, Suarez J, et al. Cholesterol: a useful parameter for distinguishing between pleural exudates and transudates. Chest 1991; 99: 1097-102.*
12. *Pachon EG, Navas IP, Sanchez JF, et al. Pleural fluid to serum cholinesterase ratio for the separation of transudates and exudates. Chest 1996; 110: 97-101.*
13. *Romero S, Candela A, Marin C, et al. Evaluation of different criteria for the separation of pleural transudates from exudates. Chest 1993; 104: 399-404.*
14. *Guleria R, Agarwal SR, Sinka S, et al. Role of pleural fluid cholesterol in differentiating transudative from exudative pleural effusion. Natl Med J India 2003; 16: 64-9.*
15. *Zannis VI, Breslow JL, Utermann G. Proposed nomenclature of apo E isoforms, apo E genotypes and apo E phenotypes. J Lipid Res 1982; 23: 911-4.*
16. *Staats BA, Ellefson RD, Budhahn LL, et al. The lipoprotein profile of chylous and nonchylous pleural effusions. Mayo Clin Proc 1980; 55: 700-4.*
17. *Rerabek JE. Low density lipoproteins and immunoglobulins in human pleural effusions. Clin Chim Acta 1977; 76: 363-9.*
18. *Raymond TL, Reynolds SA. Lipoproteins of the extravascular space: alterations in low density lipoproteins of interstitial inflammatory fluid. J Lipid Res 1983; 24: 113-9.*