The Korean Journal of Internal Medicine
Vol. 3, No. 2, July, 1988

C-Reactive Protein, Sialic Acid and Adenosine Deaminase Levels in Serum and Pleural Fluid from Patients with Pleural Effusion

Ji Woon Kim, M.D., In Ae Yang, M.D., Eun A Oh, M.D.
Young Gun Rhyoo, M.D. and Young Ho Jang, M.D.

Department of Internal Medicine, St. Columban's Hospital, Mokpo, Korea

Dong Wook Ryang, M.D. and Joo Yong Yoo, M.D.

Department of Clinical Pathology, Chunnam University Medical School, Kwang Ju, Korea

Laboratory analysis of pleural fluids is essential to determine underlying diseases. The authors evaluated the clinical significance of C-reactive protein (C-RP), sialic acid (SA), and adenosine deaminase (ADA) determinations in sera and pleural fluids from 37 patients with pleural effusion.(FP12)C-RP and sialic acid levels and ADA activities were higher in exudates than in transudates of pleural fluids. Serum and pleural fluid C-RP levels were high in patients with pyothorax. Determinations of serum sialic acid and the pleural fluid to serum ratio were useful for the differential diagnosis of pulmonary tuberculosis and malignancy. ADA activities of pleural fluid and serum are useful for the differentiation of malignancy from tuberculosis and nonspecific pyothorax. C-RP concentrations of pleural fluid correlated to serum levels. However, concentrations of sialic acid and ADA activities were not correlated to serum levels and only correlated to protein concentrations of pleural fluids.

Key Words: *C-reactive protein, Sialic acid, Adenosine deaminase, Pleural effusion*

INTRODUCTION

Laboratory analysis of the pleural fluid is essential for the diagnosis of underlying pleural diseases. In the past, differentiation between transudate and exudate was dependent on their specific gravities, protein and glucose concentration, lactate dehydrogenase (LDH) levels and differential cell counts; but recently pleural fluid to serum ratio of the protein and lactate dehydrogenase concentration, and total lactate dehydrogenase levels of the pleural fluids are more often used.[1] However, in exudates, underlying diseases are not easily differentiated by such a pleural fluid analysis, and cytologic examinations of the pleural fluids or pleural biopsy is usually specific but not sensitive enough for definitive diagnosis.[2]

In Korea, incidences of pulmonary tuberculosis are high and malignant diseases such as bronchogenic cancer are increasing in incidence.

Therefore, other diagnostic methods which easily differentiate tuberculous pleural effusion from malignant effusions are recommended.

Thus, the author and co-workers studied c-reactive protein(CRP),[3] an acute phase reactant, and sialic acid which can be elevated in various cancers and inflammatory diseases[4,5] by quantitative measurement, and measured adenosine deaminase activities, which are known to be elevated in tuberculosis,[6] typhoid fever and liver cirrhosis,[7] in order to evaluate the differential diagnostic significance of these tests on pleural effusion.

MATERIALS AND METHODS

1. Materials

We tested 73 pleural effusion and 43 serum

Adress reprint requests: J: Woon Kim, M.D., Department of Internal Medicine, St. Colomban's Hospital, 97 San Jung Dong, Mokpo, 530-350, Korea

specimens obtained from patients with pleural effusions who had been admitted to Chunnam Medical School Hospital and St. Colomban's Hospital. Diseases were classified as malignancy, pulmonary tuberculosis, pyothorax and transudate groups by cytologic, histologic, and bacteriologic culture examinations in conjunction with clinical findings. The normal control group was 12 adult men and women who had no abnormal findings on their biochemical serum examinations (Table 1)

METHODS

We tested the pleural fluid and serum specimens within 3 days after sampling. All speciments were refrigerated at -20°C.

C-RP measurement: C-RP level was determined quantitatively with a turbidimetric immunoassay kit.[8]

Sialic acid measurement: Sialic acid levels was measured by an enzymatic method employing neuraminidase.[9]

Adenosine deaminase (ADA) acitivity measurement: ADA was measured by the colorimetric assay of Guisti.[10]

Table 1. Diseases and Number of Specimens

Diseases	No. of specimens	
	pleural fluid	serum
Malignancy*	22	11
Tuberculous	29	10
Pyothorax	9	6
Other diseases**	13	10
Control	0	12
Total	73	49

*: includes squamous cell carcinoma (7), adenocarcinoma (10), mucoepidermoid carcinoma (1), lymphoma (2), oat cell carcinoma (1) and osteogenic sarcoma (1).

**: includes trauma (3), hepatoma (5), chronic renal failure (2) and cirrhosis (3).

RESULTS

1. C-RP, Sialic Acid Levels and ADA Activities in Transudates and Exudates

We differentiated the transudates by the standard methods, namely pleural fluid to serum ratio of total protein and LDH levels, total LDH levels of the pleural fluids, and total leukocyte counts of the pleural fluids (in exudates: protein ratio >0.5, LDH ratio >0.6, total LDH level >200 IU/L, and total leukocyte counts >1000/cu mm). The C-RP, Sialic acid and ADA levels in the transudates (mean ± SD) were 0.18 ± 0.21 mg%, 29.60 ± 32.43 mg% and 20.68 ± 11.30 IU/L respectively, but in exudates, the C-RP level was 4.86 ± 5.96 mg%, the Sialic acid level was 72.40 ± 29.83 mg%, and ADA activity was 75.89 ± 59.22 IU/L, indicating a significant increase in the exudates ($p<0.05$, <0.01). (Table 2)

2. C-RP Levels in the Pleural Fluids and Serums

C-RP levels of the sera of patients with pleural effusion were higher compared to the normal control group (0.21 ± 0.33mg%), and were significantly increased in the exudates ($p<0.01$). On the other hand, C-RP levels of the pleural fluids and sera were especially high in the pyothorax among patients with pleural effusion, with results of 9.17 ± 7.32mg% in the pleural fluid and 12.38 ± 5.51 mg% in the serum, and were more useful for the differentiation of malignancy from tuberculosis ($p<0.05$). (Table 3)

3. Sialic Acid Levels in the Pleural Fluids and Serums

Sialic acid levels were significantly higher in the exudates secondary to malignancy and tuberculosis than in the transudates ($p<0.05$). Sialic acid levels in malignancies were 65.20 ± -26.29 mg% in the pleural fluids and 127.79 + 70.28 mg% in the sera which were comparatively higher than the serum levels in tuberculosis (86.90 ± -36.15 mg%) with a

Table 2. Camparison of C-Reactive Protein (CRP), Sialic acid(SA), and Adenosine Deaminase (ADA) Activities Between Transudate and Exudate of Pleural Fluid (mean ± SD)

	No.	CRP (mg/dl)	SA (mg/dl)	ADA (IU/L)
Transudate	4	0.18 ± 0.21	29.60 ± 32.43	20.68 ± 11.30
Exudate	50	4.86 ± 5.96	72.40 ± 29.83	75.79 ± 59.22
P-value		<0.01	<0.05	<0.01

significant difference in their pleural fluid to serum ratios ($p<0.05$). (Table 4)

4. ADA Activities in the Pleural Fluids and Sera

ADA activities in the pleural fluids and sera were not significantly increased in malignancies compared to the normal control value (22.40 ± -8.40 IU/L), but ADA activities were high in the sera and pleural fluids, especially in tuberculosis ($p<0.01$, 0.05) (Table 5).

5. Presumptive sensitivity of ADA Assay in the Pleural Fluids Between Malignancy and Tuberculosis

Using an ADA value of pleural fluids in malignancies of 41.01 IU/L, determined by taking the mean (24.12) + 1SD (16.89) as the presumptive differential value, 19 of 22 malignant effusions were below this value and 25 of 28 tuberculosis effusions were above it. Thus, the calculated sensitivity was 86.4%, specificity was 89.3% and efficiency was 88.0%. Therefore, we found that these values were very efficient in differentiating between malignant and tuberculous effusions (Table 6).

6. Correlation of C-RP, Sialic Acid, Adenosine Deaminase Levels and Other Parameters of Pleural Fluid Examination (Correlation Efficient)

The C-RP levels of the pleural fluids showed positive correlation to serum C-RP levels and LDH

Table 3. Comparison of Pleural Fluid and Serum Levels (mean ± SD) of C-RP (mg/dl) in Various Diseases

Group (N1, N2) / Sample	Malignancy (22, 11)	Tuberculosis (28, 10)	Pyothorax (9, 6)	Other disease (13, 10)	Control (0, 12)
pleural fluid	3.75 ± 4.39	3.83 ± 5.84	9.17 ± 7.32[a,c]	2.56 ± 2.86[c]	
Serum	6.41 ± 7.23[h]	5.07 ± 3.58[h]	12.38 ± 5.51[e,g,h]	8.46 ± 14.56	0.21 ± 0.33
Ratio pleural fluid serum	0.58 ± 0.48	1.99 ± 2.68	0.94 ± 0.72	0.83 ± 0.66	

N1 : Number of tested pleural fluid
N2 : Number of tested serum samples
a : Significant difference from malignancy group, $p<0.05$
b : " " $p<0.01$
c : Significant difference from tuberculosis group, $p<0.05$
d : " " $p<0.01$
e : Significant difference from pyothroax group, $p<0.05$
f : " " $p<0.05$
g : Significant difference from serum control, $p<0.05$
h : " " $p<0.01$

Table 4. Comparison of Pleural Fluid and Serum Levels (mean ± SD) of Sialic Acid (mg/dl) in Various Diseases

Group (N1, N2) / Sample	Malignancy (22, 11)	Tuberculosis (29, 10)	Pyothorax (9, 6)	Other disease (13, 10)	Control (0, 12)
Pleural fluid	65.20 ±26.29	82.98 ±67.44	72.97 ±52.53	35.46[b,d,e] ±27.99	
Serum	127.79[h] ±70.28	86.90[e,g] ±36.15	113.82[h] ±29.59	55.57[h,c,f] ±23.18	57.96 ±5.09
Radio pleural fluid serum	0.60 ±0.33	1.13[e] ±0.85	0.68 ±0.77	0.73 ±0.47	

Abbreviations are the same as in Table 3

Table 5. Comparison of Pleural Fluid and Serum Levels (mean ± SD) of Adenosine Deaminase (IU/L) in Various Diseases

Group (N1, N2) / Sample	Malignancy (22, 11)	Tuberculosis (28, 10)	Pyothorax (9, 6)	Other disease (13, 10)	Control
Pleural fluid	35.18 ±54.57	94.74[b] ±46.3	87.59[a] ±54.31	30.04[d,e] ±20.97	
Serum	28.40 ±19.18	43.36[e] ±34.17	45.47[A] ±25.42	43.37[a,b] ±18.05	22.40 ±8.40
Radio pleural fluid serum	1.21 ±1.33	3.68[a] ±2.73	1.47[c] ±0.54	0.84[d,e] ±0.48	

Abbreviations are the same as in Table 3

Table 6. Presumptive Sensitivity of Adenosine deaminase (ADA) Assay in Pleural fluid Between Malignancy and Tuberculosis

	No. of cases		Total number of cases
	less than 41.01 IU/L*	More than 41.01 IU/L	
Malignancy	19	3**	22
Tuberculosis	3	25	28
Total	22	28	50

*: ADA value of pleural fluid in malignant disease, determined by mean (24.12) + 1SD (16.89)
**: Includes malignant lymphoma (2) and metastatic adenocarcinoma (1)
Sensitivity: 86.4% (19/22)
Specificity: 89.3 (25/28)
(+) Predictive value: 86.4% (19.22)
(−) Predictive value: 89.3% (25/28)
Efficiency 88.0% (44/50)

Table 7. Correlation of C-RP, Sialic Acid (SA), Adenosine Deaminase (ADA) Levels and Other Parameters of Pleural fluid Examination (Correlation Coefficient)

Sample	Pleural fluid			Cell		Serum		
# / Test	Protein (N=57)	Sugar (N=57)	LDH (N=54)	Neut. (N=57)	Lymph	C-RP (N=31)	SA (N=32)	ADA (N=32)
C-RP	0.2139	−0.2017	0.4171***	0.0545	−0.0421	0.4631	0.0104	0.1559
SA	0.3359*	−0.0988	0.1369	−0.1588	−0.0147	−0.0713	0.1855	0.2339
ADA	0.4459**	−0.1208	0.1226	0.0917	0.1512	0.0334	−0.1421	0.2327

#: level of pleural fluid
*: $p<0.05$ **: $p<0.01$

levels of the pleural fluids. The sialic acid and ADA levels didn't show a correlation to the pleural fluids and sera, but showed positive correlation to the protein levels of the pleural fluids only. (Table 7)

DISCUSSION

Since discovery of the C-RP by Tillet and Francis,[11] it has been used as an adjunctive diagnostic

method for the various inflammat diseases, tissue trauma and malignant diseases, or as a useful means of clinical course observation.[3] However, it was usually measured semiquantitatively by the latex agglutination method and studies using it were largely restricted to its sera level analysis, and those using body fluids such as pleural effusion were rare. In this study, we tested pleural effusions and sera of patients with pleural effusion by quantitative measurement of the C-RP levels using a turbidimetric immunoassay which is more rapid and accurate.

C-RP levels of the pleural fluids and sera were higher in exudative pleural diseases than in transudative pleural diseases, and especially higher in diseases causing pyothorax than in malignancies and tuberculous diseases. This result was correlated to that of Cho and his coworkers' study[13] in which sera levels of C-RP were higher in non-specific inflammatory diseases than in tuberculous peritonitis.

Sialic acid is present as a component of cellular membrane glycoproteins, glycolipids,[15] and sera glycoproteins. Several studies[4,5,16,17] showed that sialic acid was increased in malignant and inflammatory diseases, shock, trauma, myocardial infarction, diabetes mellitus and liver diseases. But these studies were done by measurement of sera sialic acid levels only and pleural fluid study[13] on sialic acid was rare.

In this study, sialic acid measurement in the pleural fluids and sera was useful for differentiating exudate from transudate, and was helpful in distinguishing tuberculous from malignant effusion, but not in differentiating nonspecific pyothorax. These findings were not correlated to the results of Cho and his co-workers' study[13] showing that sialic acid measurement in ascites was helpful in distinguishing exudate from transudate, but not in differentiating similar ascitic fluids of various diseases.

ADA is an enzyme which participates in the catabolism of nucleic acids and nucleoproteins. It is distributed widely in tissues and blood of mammals and in the reticuloendothelial system, spleen and digestive tract tissue (duodenum, stomach, small intestine, cecum) of man.[18]

Giblett et al[19] reported a deficiency of ADA in patients with cellular immune deficiency. Meuwissen and Pollara[20] reported thymus regression, lymphocyte disappearance and T-lymphocyte functional deficiency associated with ADA deficiency, and they suggested that ADA activity was closely related to immune function and differential proliferation of lymphocytes. Kim et al[21] reported decreased ADA activities in lepromatous leprosy patients with cellular immune disturbances.

ADA activities in sera have been known to be increased in typhoid fever,[22] infectious mononucleosis,[23] viral hepatitis, liver cirrhosis and hepatoma.[7,24,25] On the other hand, Blake and Berman,[26] Martinez-Vazquez,[27] Piras et al,[28] Cha et al[25] have reported higher ADA activities in tuberculous ascites, pleural effusions and CSF than in other diseases, as in Cho's report[13] on ascites and Cho's report[23] on CSF.

In this study, ADA activities in pleural fluids were higher in exudates than in transudates, as were C-RP and sialic acid levels. Cha et al[25] suggested that an increase in ADA activity might not be characteristic of exudates because of non-correlation of ADA activities to protein and LDH levels measured in exudative ascites. But in this study, ADA activities, C-RP and sialic acid levels measured in the pleural fluids were higher in exudates compared to transudates, and sialic acid levels and ADA activities of the pleural fluids were positively correlated to the protein concentrations of the pleural fluids. Thus, ADA activities were thought to be useful in evaluating exudates.

In this study, ADA activities were increased in tuberculosis rather than malignancy, which was helpful in differentiating malignancy from tuberculosis, but not in differentiating tuberculosis from nonspecific pyothorax. This fact causes problems to arise in the explanation that ADA activities are increased by increases in lymphocyte numbers in serum or subpopulations of lymphocytes in the pleural fluid.[13,20,25]

When we used a value of 41.01 IU/L, the mean of ADA activities + 1 SD, in malignancy as a differential value for the differentiation of tuberculosis from malignancy in pleural fluids, 19 of 22 malignant specimens were below this value and 25 of 28 tuberculous ones were above it, and thus the calculated sensitivity, specificity, and efficiency are all above 85%.

In the correlation of C-RP, sialic acid, ADA levels and other parameters of pleural fluid examinations, C-RP levels of pleural fluids showed positive correlation to serum C-RP and LDH levels of pleural fluids. Alternatively, sialic acid and ADA levels showed only correlation to protein concentrations of the pleural fluids, which suggested some correlation to the characteristics of exudates. The fact that sialic acid levels and ADA activities in the pleural fluids and sera have no correlation with each other suggested that sialic acid and C-RP levels in pleural fluids might not have merely entered from the serum, but they seem-

ed to have some correlation to lymphocytes infiltration caused by pleural disease itself or mesothelial reactions.

REFERENCES

1. Light RW, MacGregor MI, Luchsinger PC, Ball WC jr: *Pleural effusion: The diagnostic separation of transudate and exudate. Ann Int Med 77: 507, 1972*
2. Brody JS: *Disease of the pleura, mediastinum, diaphragm and chest wall. Cecil textbook of medicine, 17th ed. Philadelphia W.B. Saunders p 450, 1985*
3. Pepys MG: *C-reactive protein fifty years on. Lancet 1:653, 1981*
4. Araki E: *Sialic acid in malignancy. Clin Pathol 33:19, 1985*
5. Taoka Y: *Biochemistry and clinical significance of sialic acid. Clin Pathol 54:2, 1983*
6. Piras MA, Gakis C, Budroni M, Andreon G: *Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. Med J 23:1751, 1978*
7. Raczynska J, Jonas S, Krauwczynski J: *Diagnostic values of adenosine deaminase in some liver disease. Clin Chem Acta 13:151, 1966*
8. Instruction manual for turbidimetric immunoassay: *C-RP. Nissui Co, Tokyo, 1986*
9. Instruction manual for determination of sialic acid: *Kyokuto Co, Tokyo, 1986*
10. Giusti G: *Adenosine deaminase: Method of enzymatic analysis. 2nd ed. Verlag Chemie, Wienheim and Academic Press, New York, p 1092, 1974*
11. Tillet Ws, Francis T Jr: *Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J Exp Med 52:561, 1930*
12. Corrall CJ, Pepple JM, Moxon ER, Hughes WT: *C-reactive protein in spinal fluid of children with meningitis. J JPaediatr 99:365, 1981*
13. Cho JH, Ryang DW, Yoo JY: *Significance of C-RP, sialic acid and adenosine deaminase assay in patients with ascites. Kor J Clin Pathol 7:9 1987*
14. Otsuji S, Shibata H, Umeda M: *Turbidimetric immunoassay of serum C-reactive protein. Clin Chem 28: 2121, 1982*
15. Meezan T,et al: *Comparative studies of the carbohydrate- containing membrane components of normal and virus-transformed mouse fibroblasts. II Seperation of glycoproteins and glycopeptides by sephadex chromatography. Biochemistry 8:2518, 1969*
16. *Walkowiak H, et al: Horizontal circular chromatography of sialic acids from serum certain mammalian species. Bull Acad Pol Ser Sci Bio1 16:97, 1968*
17. Son HC: *A study on level of serum sialic acid in patients with various cancer. J Pusan Med College 25: 217, 1985*
18. Martin B, Van der Weyden, William NK: *Human adenosine deaminase distribution and properties. J Bio Chem 251:5448, 1976*
19. Giblett ER, Anderson JE, Cohen F, Pollara B, Meuwissen HJ: *Adenosine deaminase deficiency in two patients with severely impaired cellular immunity. Lancet II: 1067, 1972*
20. Meuwissen HJ, Pollara B: *Combined immunodeficiency and inborn errors of purine metabolism. Blut 37:173, 1978*
21. Kim JY, Choi YS: *Adenosine-Deaminose-Aktivitat Bei Leprapatienten. Hautarzt: Supplementum, 34:365, 1983*
22. Kang SD, Kim YJ, Lee SY, Ryoo UH, Kim CS: *A study on adenosine deaminase activity in normal and typhoid fever. Kor J Int Med 22:612, 1979*
23. Koehler H, Benz EZ: *Serum adenosine deaminase: Methodology and application. Clin Chem 8:133, 1962*
24. Lee SJ, Ji MY, Park SM, Kim JS: *On serum adenosine deaminase activity in liver diseases. Kor J Int Med p 852, 1978*
25. Cha KS, Park BG, Um SJ, Rhee IS, Hur SH, Shin SH, Park SM, Kim CS: *A study on adenosine deaminase activity in pleural effusions and ascites. Kor J Int Med 25: 364, 1982*
26. Blake J, Berman P: *The use of adenosine deaminase assay in the diagnosis of tuberculosis. S Afr Med J 62: 19, 1982*
27. Martinez-Vazquez, JM, Ocana I, Ribera E, Segura RM, Pascual C: *Adenosine deaminase activity in the diagnosis of tuberculosis peritonitis. Gut 27: 1049, 1986*
28. Piras MA, Gakis C: *Adenosine deaminase activity in CSF: an aid to differential diagnosis. Enzyme 14: 311, 1973*
29. Cho MK, Ryang DU: *C-RP, sialic acid and adenosine deaminase determinations in serum and CSF of meningitis patients. Kor J Paediatr (being printed)*