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Ferritin Assay in Malignant Pleural Effusion

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In order to assess the usefulness of ferritin as a tumor marker, the authors measured and analyzed pleural fluid and serum ferritin concentrations by double antibody radioimmunoassay method in 20 patients with malignant pleural effusion, and in 39 patients with benign pleural effusion. Serum ferritin levels were also measured in a control group of 20 healthy people.

The results obtained are summarized as follows:

1) Pleural fluid ferritin levels in the malignant pleural effusion group were significantly higher ($p<0.001$) than those of the benign pleural effusion group.

2) As one of the criteria in differentiating between malignant and benign pleural effusion, the differentiating pleural fluid ferritin level was set at 2,000 ng/ml, a specificity up to 75.0% and sensitivity of 89.7% could be obtained.

3) Serum ferritin levels in the malignant pleural effusion group were significantly higher ($p<0.001$) than those in the control group.

4) There was no statistically significant correlation between pleural fluid and serum ferritin levels in the malignant pleural effusion group.

From the above results, it can be concluded that it is possible to use pleural fluid ferritin levels as a tumor marker.

Key Words: *Pleural effusion, Ferritin, Radioimmunoassay*

INTRODUCTION

Ferritin, an iron containing, iron storage protein, exists in the human liver, spleen and bone marrow. Ferritin levels closely reflect the amount of iron in the body[1~5], and the serum ferritin test is known to be the most accurate mothod for the assessment of the amount of body iron stores. Moreover, serum ferritin is known to be increased not only in various liver diseases and inflammatory diseases but also with malignant tumors[1,4,6~12], and there have been attempts to use ferritin as a tumor marker[1].

Differential diagnosis of pleural effusion is one of the most frequent difficulties in the field of pulmonology. A significant portion of pleural effusions are caused by malignant tumors[13] and differentiation those caused by benign tumors just by thoracentesis and clinical manifestation is often difficult and usually requires cytologic or histologic examination as well. Malignant pleural effusion can be diagnosed in about 60% of patients by cytologic examination and in about 40 ~60% by pleural biopsy. When both of the above two methods are performed, diagnosis can be confirmed in up to 60~90% of cases[14]. Even when both cytologic and histologic examinations are carried out together, the differential diagnosis still remains difficult in some cases. It has been reported that measurement of orosomucoid, β_2-microglobulin or carcinoembryonic antigen (CEA) in the pleural fluid can be used as an additional examination to assist in the differential diagnosis of pleural effusion[15~18].

This is a report of ferritin assays in both serum and pleural fluid in patients with pleural effusions to see whether ferritin could be used as a reliable marker to differentiate between benign and malignant causes or not. The results were analyzed in

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regard to diagnostic significance and use as criteria for differential diagnosis.

SUBJECTS AND METHODS

1. Subjects

The study was done on 59 patients who were admitted to Pusan National University Hospital between February 1985 and August 1986 because of pleural effusion. All had undergone extensive investigations in order to diagnose the causes of the effusion. Among them, there were 20 cases with malignant pleural effusion confirmed by case history, clinical symptoms, chest roentgenography and computerized tomography, cytologic examination of sputum and pleural exudate, pleural biopsy, lymph node biopsy and percutaneous transthoracic needle aspiration and biopsy. There were 39 cases of benign pleural effusion confirmed by pleural biopsy, and smear and culture of sputum for tubercule bacilli and which also included cases that showed an improvement on antituberculosis chemotherapy.

The control group consisted of 20 healthy subjects, 10 of each sex. Among the 20 cases of malignant pleural effusion, the most common type was primary bronchogenic carcinoma with 14 cases; 6 adenocarcinoma, 5 squamous cell carcinoma, 2 small cell carcinoma, and 1 mucoepidermoid tumor. The remainder were metastatic carcinoma patients, 2 of known origin, namely 1 case each from breast and stomach and 4 of unknown origin, namely, 3 cases of adenocarcinoma and 1 case of undifferentiated carcinoma.

Table 1. Etiologies of Malignant Pleural Effusion

Diagnosis	No. of patients	
Lung cancer	14	
Adenocarcinoma		6
Squamous carcinoma		5
Small cell carcinoma		2
Mucoepidermoid tumor		1
Metastatic cancer	6	
Breast (ductal cell carcinoma)		1
Stomach (adenocarcinoma)		1
Unknown origin		
Adenocarcinoma		3
Undifferentiated carcinoma		1
Total		20

Among the 39 cases of benign pleural effusion, there were 26 with tuberculous pleurisy, 3 with liver cirrhosis with ascites, 3 with nephrotic syndrome, 2 with chronic renal failure, 2 with congestive heart failure, and 1 case each of pneumonia, empyema, and interstitial lung disease.

2. Methods

The measurements of the pleural fluid and serum ferritin concentrations were made by using a double antibody radiolabeled ^{125}I-ferritin radioimmunoassay kit produced by Diagnostic Products Corporation of U.S.A.. When the concentration exceeded the level of 1,000 ng/ml, dilution measurements were used instead, reading up to 10,000 ng/ml.

RESULTS

1) There was a significant difference in mean pleural fluid ferritin levels between benign and malignant pleural effusions with levels of 894.4± 729.9 ng/ml and 2656±2194.2 ng/ml respectively ($p<0.001$) (Table 3, Fig. 1 and 2).

When the mean serum ferritin levels were

Table 2. Etiologies of Benign Pleural Effusion

Diagnosis	No. of patients
Tuberculous	26
Liver cirrhosis	3
Nephrotic syndrome	3
Chronic renal failure	2
Congestive heart failure	2
Pneumonia	1
Empyema	1
Interstitial lung disease	1
Total	39

Table 3. The Mean Values of Pleural Fluid & Serum Ferritin in Benign & Malignant Pleural Effusion

Group	Pleural fluid (ng/ml)	Serum (ng/ml)
Benign effusion	894.2 ± 729.9	160.8 ± 80.6
Malignant effusion	2,656.4 ± 2,194.2	193.3 ± 60.2
P value	P < 0.001	NS

Values are expressed as mean ± SD.
NS : Not significant

compared between the 2 groups of pleural effusion, there was no significant difference, benign effusion 160.8±80.6 ng/ml and malignant effusion 193.3±60.2 ng/ml, but when these levels were compared separately to the mean serum ferritin level of the control group, 103.9±56.0 ng/ml a significant difference was seen, $p<0.01$ with benign effusion and $p<0.001$ with malignant effusion (Table 4 and Fig. 2).

2) In the malignant pleural effusion group pleural fluid ferritin levels in the 14 cases of primary bronchogenic carcinoma were compared to levels in 6 cases of metastatic cancer. There was no difference between the two groups, being 3105.8±2379.5 ng/ml in primary bronchogenic carcinoma and 1554.8±1075.8 ng/ml metastatic cancer.

There was also no difference in serum ferritin levels, being 192.4±54.7ng/ml in primary bronchogenic carcinoma and 195.2±71.4 ng/ml in metastatic cancer (Table 5).

3) In the malignant pleural effusion group the pleural fluid and serum ferritin levels were compared between 10 cases of adenocarcinoma and 10 cases of non-adenocarcinoma. The mean pleural fluid ferritin level in adenocarcinoma group was 2491.1±1748.0 ng/ml and in non-adenocarcinoma group was 2789.9±2555.7 ng/ml. The mean serum ferritin level in adenocarcinoma was 181.4±55.3 ng/ml and in non-adenocarcinoma was 205.1±62.5 ng/ml. No statistically significant difference was present between the 2 groups in either study (Table 6).

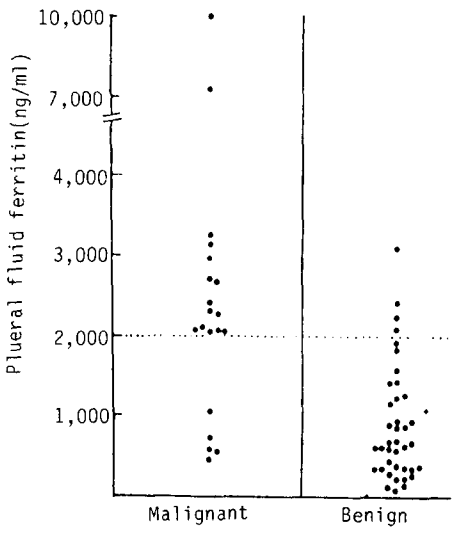

Fig. 1. *Levels of pleural fluid ferritin in patients with malignant and benign pleural effusion.*

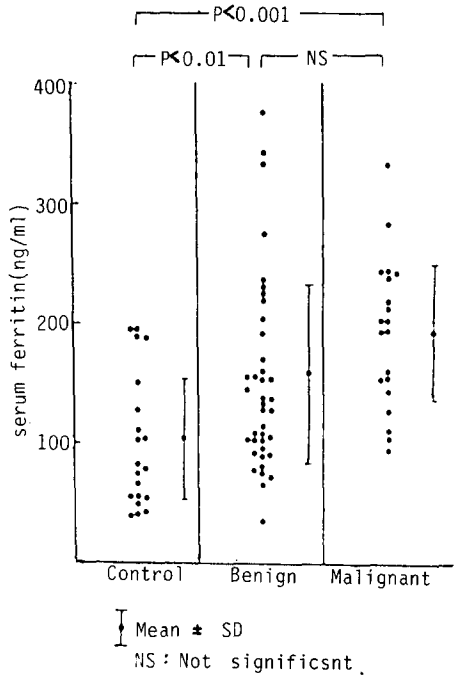

Fig. 2. *Comparison of serum ferritin levels in normal control group & patients with benign & malignant pleural effusion.*

Table 4. The Mean Values of Serum Ferritin in Control Group, Benign & Malignant Pleural Effusion

Group	Serum ferritin (ng/ml)	P value
Control	103.9 ± 56.0	
Benign effusion	160.8 ± 80.6	P < 0.01
Malignant effusion	193.3 ± 60.2	P < 0.001

Values are expressed as mean ± SD.

Table 5. The Mean Values of Pleural Fluid & Serum Ferritin in Primary & Metastatic Lung Cancer

Group	Pleural fluid (ng/ml)	Serum (ng/ml)
Primary	3,105.8 ± 2,379.5	192.4 ± 54.7
Metastatic	1,554.8 ± 1,075.8	195.2 ± 71.4
P value	NS	NS

Values are expressed as mean ± SD.
NS : Not significant

4) There was no statistically significant correlation between mean pleural fluid ferritin and serum ferritin levels in the malignant pleural effusion group ($r = -0.17$) (Fig. 3).

5) The distribution of ferritin levels in pleural fluid was studied. In the malignant pleural effusion group, the minimum level was 498 ng/ml and the maximum level 10,000 ng/ml. There were 4 cases (20.0%) ranging between 101-1,000 ng/ml, 1 case (5.0%) between range of 1,001-2,000 ng/ml, 13 cases (65.0%) between the range of 2,001-5,000 ng/ml, and 2 cases (10.0%) between the range of 5,001-10,000 ng/ml. In the benign pleural effusion group, the range was between a minimum level of 56 ng/ml and a maximum level of 3014 ng/ml. There were 3 cases (7.7%) between the range of 1-100 ng/ml, 24 cases (61.5%) between the range of 101-1,000 ng/ml, 8 cases (20.5%) between the range of 1,001-2,000 ng/ml and 4 cases (10.3%) between the range of 2,001-5,000 ng/ml (Table 7 and Fig. 1).

6) According to these results when a pleural fluid ferritin level of 2,000 ng/ml was taken as the level to differentiate between malignant and benign effusions the ferritin level was above this point in 15 out of the 20 cases of malignant effusion, showing a sensitivity of 75.0% and specificity of 89.7%.

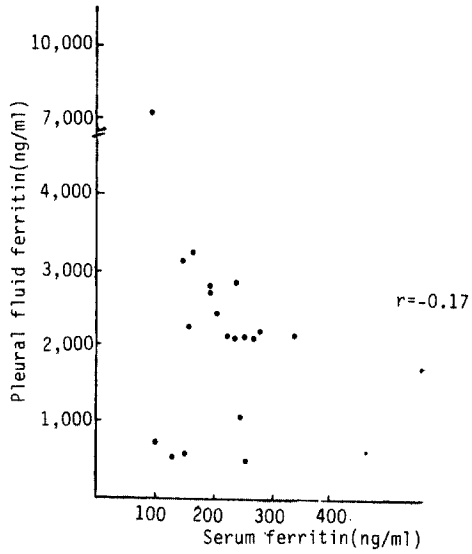

Fig. 3. *Relationship between pleural fluid & serum ferritin in patients with malignant pleural effusion.*

Table 6. The Mean Values of Pleural Fluid & Serum Ferritin in Adenocarcinoma & Non-adenocarcinoma

Group	Pleural fluid (ng/ml)	Serum (ng/ml)
Adenocarcinoma	2,491.1 ± 1,748.0	181.4 ± 55.3
Non-adenocarcinoma	2,789.9 ± 2,555.7	205.1 ± 62.5
P value	NS	NS

Values are expressed as mean ± SD.

Table 7. Distribution of Pleural Fluid Ferritin in Patients with Malignant & Benign Effusion

Pleural fluid ferritin (ng/ml)	No. of patients with malignant effusion (%)	No. of patients with benign effusion (%)
1 – 100		3 (7.7)
101 – 1,000	4 (20.0)	24 (61.5)
1,001 – 2,000	1 (5.0)	8 (20.5)
2,001 – 5,000	13 (65.0)	4 (10.3)
5,001 – 10,000	2 (10.0)	
Total	20 (100.0)	39 (100.0)

DISCUSSION

Ferritin was first separated from the liver and spleen of the horse by Laufberger in 1937[19]. Ferritin is one of the most essential iron storage proteins and can be detected in most tissues but is mainly concentrated in liver, spleen, and bone marrow. It forms 15~20% of total body iron content[20,21].

Apoferritin which surrounds the outer wall of the ferritin molecule, is synthesized mainly from free polysome of the liver and partially from the endoplasmic reticulum[22]. Iron injected into the body stimulates the synthesis of apoferritin and is transported by transferrin to be utilized in the formation of iron micelle in a form of Fe^{++}. To this iron micelle 24 apoferritins bond together and form a globular shaped ferritin[23,24]. 5,000 ions of iron bond to one ferritin molecule and the molecular weight of the ferritin molecule is 450,000[1].

Ferritin consists of many different kinds of isoferritin, but may be divided broadly into two categories of basic isoferritin and acidic isoferritin according to it's isoelectric point, since each tissue ferritin has a different motility under electrophoresis and isoelectric focusing[25~28]. It has been shown that basic isoferritin exists mainly in the

liver and spleen and forms most of the serum ferritin, whereas acidic isoferritin is known to be present in heart, kidney, pancreas and placenta[20,29~31]. A rise of the serum ferritin level[6,10~12] and changes in its composition[32,33] in various malignant tumors have been reported, and since both tissue and serum ferritin in malignant tumors are known to be acidic isoferritin, there have been attempts to use acidic isoferritin as a tumor marker[11,31,33]. Maxim et al.[34] reported an increase in ferritin levels in lung cancer tissue. Serum ferritin, together with carcino-embryonic antigen (CEA), has been reported to be useful in indicating tumor recurrence and thus in postoperative follow up[35,36].

In this experiment there was no statistically significant difference between serum ferritin levels of the malignant pleural effusion group of 193.3± 60.2 ng/ml and the benign effusion group of 160. 8±80.6 ng/ml. However when the levels of each group were compared separately to the serum ferritin levels of the control group, 103.9±56.0 ng/ml, both groups showed a statistically significant difference, $p<0.001$ and $p<0.01$ respectively. These results are compatible with other reports that serum ferritin levels increase both in various inflammatory diseases and in malignant tumors[6,9~12].

As the pleural fluid ferritin levels in malignant and benign effusions showed a statistically significant difference, 2656.4±2194.2 ng/ml and 894.2± 729.9 ng/ml respectively ($p<0.001$), the possibility that pleural fluid ferritin measurement could be used as a tumor marker to assist in the differentiation between malignant and benign pleural effusion was investigated.

If the differentiating level for pleural fluid ferritin was set at 2,000 ng/ml, the ferritin level was positive in 15 out of the 20 cases of malignant pleural effusion indicating a sensitivity of 75.0% and specificity of 89.7%. When the above result was compared to the reports of Millano[37] and Jung et al.[38] their differentiating levels were similar, at 2,000 ng/ml and 1,900 ng/ml respectively. However, their results of sensitivity were only 34% and 69% respectively, much lower than the results of this study. The authors are of the opinion that the disparity in sensitivity could be due to the difference in the subjects studies and to the methods used in measuring ferritin levels.

When pleural fluid CEA, was used as a tumor marker, James et al.[39] reported that high levels of pleural fluid CEA were present when the cause of the pleural effusion was adenocarcinoma.

However, in this experiment, there was no significant difference in levels of pleural fluid ferritin between adenocarcinoma and non-adenocarcinoma group. Moreover, there was also no statistically significant difference between primary lung cancer and metastatic cancer groups.

The possible causes of the rise in ferritin levels in malignant pleural effusions can be listed as follows: first, an influx of serum ferritin into the plerual fluid, secondly, hemolysis of erythrocytes and thus release of ferritin from erythrocytes, and most of the malignant pleural effusions are bloody, and thirdly, as malignant tumor invades the pleura, increased synthesis of ferritin from malignant tumor cells and an influx of ferritin from the surrounding inflammatory tissues occurs[38].

The authors only measured plerual fluid and serum ferritin levels in order to make a differential diagnosis of malignant pleural effusion, however, if the already widely used CEA as a tumor marker[17,18] had been measured at the same time, probably, results of a higher sensitivity and specificity would have been obtained.

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