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Pleural Fluid Lysozyme in Human Disease[1] (39344)

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Despite the availability of knowledge concerning the pathophysiology and clinical usefulness of lysozyme (EC 3.2.1.17, mucopeptide *N*-acetylmuramylhydrolase, muramidase) of serum, urine, cerebrospinal fluid, middle ear fluid, amniotic fluid, genital secretions, breast milk, and synovial fluid in the diagnosis of malignant and infectious disorders (1-9), the content and source of lysozyme in pleural fluid remain poorly understood. In a preliminary study of a patient with recurrence of acute myelocyte leukemia involving pleura while bone marrow and blood were in remission, we found high lysozyme activity in the pleural fluid (10). This observation raised the possibility that measurement of the enzyme in pleural fluid might be useful in the diagnosis of pleural leukemia and other malignancies. Therefore, a prospective study was conducted to explore the potential diagnostic value of the content of pleural fluid lysozyme and to evaluate the source of the enzyme in the pleural fluid.

Materials and methods. Pleural fluid was obtained by thoracentesis from 59 patients admitted to University Hospitals. The pleural effusion was due to malignant neoplastic diseases in 37 patients and nonmalignant diseases in 22. The neoplastic group was composed of breast carcinoma in 7, lung carcinoma in 9, malignant lymphoma (including Hodgkin's disease and non-Hodgkin's lymphomas) in 9, acute myeloblastic leukemia in 2, undifferentiated carcinoma in 3, and leiomyosarcoma, carcinoid, mesothelioma, Waldenstrom's macroglobulinemia, malignant melanoma, chronic lymphocytic leukemia, and adenocarcinoma of the colon each in 1. The diagnosis of the malignancy was based on tissue biopsy in all patients. The non-neoplastic group included congestive heart failure in 10, liver cirrhosis in 3, pulmonary tuberculosis in 2, and miscellaneous conditions in 7. Paired serum samples were available from 50 of the 59 patients. These were obtained simultaneously or within 24 hr of the thoracentesis. The study was conducted prospectively. In addition to the routine diagnostic work-up, a protocol was designed to include the following determinations in each patient: (i) pleural fluid lysozyme, albumin, total protein, lactic dehydrogenase, granulocyte, total white blood cell, culture for bacteria and fungi, and cytology; and (ii) serum lysozyme, albumin, total protein, lactic dehydrogenase, white cell count, and differential. The differentiation of exudate and transudate was based on protein concentration and the ratio of pleural fluid and serum lactic dehydrogenase activity (11).

Lysozyme activity was measured by a modification (12) of a turbidometric method using egg white standard (13). The change in optical density at 20° of a cell-wall suspension of micrococcus lysodeikticus (Difco Laboratories, Detroit, Michigan) was measured at 540 nm on a recording spectrophotometer (Perkin-Elmer Coleman Instruments Division, Maywood, Illinois) at a chart speed of 20 mm/min. Initial optical density of the substrate was constituted to 0.91 ± 2 in phosphate buffer at pH 6.2. The assay is linear between 2.5 and 25 μg/ml. Samples with activity greater than 25 μg/ml were diluted. Normal serum lysozyme determined in our laboratory on 21 controls was 7.4 ± 0.43 μg/ml (mean $\pm$ standard error). Purified human lysozyme standards give values in our laboratory 0.30 times those obtained using egg white lysozyme. Protein, white blood count, lactic dehydrogenase, creatinine, and cytology were performed by the University Hospitals Laboratory Service using standard techniques.

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Results. The distribution of pleural fluid lysozyme activity in 59 patients with malignancies and other medical diseases is shown in Fig. 1. The mean pleural fluid lysozyme activity was not significantly different among various disorders studied. There was no significant difference between the malignant effusions (12.17 ± SEM 1.50) and nonmalignant effusions (12.95 ± 1.82). Likewise there was no difference between transudates (11.86 ± 1.39) and exudates (14.23 ± 2.22).

The correlation between the pleural fluid and serum lysozyme concentrations in paired samples from 50 patients was high with a correlation coefficient (r) of 0.79 (Fig. 2). In contrast, the correlation between pleural fluid lysozyme levels and pleural fluid granulocyte concentrations (available on 35 patients) was low with r = 0.37. Similar relationships were noted when the comparisons were made only for those patients whose pleural effusion was due to malignant disease. There was a high correlation between pleural fluid and serum lysozyme concentrations (r = 0.80) and low correlation between pleural fluid enzyme and granulocyte concentrations (r = 0.35).

Experiments on four pleural fluid samples performed by mixing fluid (20–40%) with a lysozyme standard revealed no evidence of a naturally occurring inhibitor in the pleural fluids.

Discussion. The present study shows that the pleural fluid lysozyme activity correlated well with simultaneous serum activity. Furthermore, the activity of the enzyme had poor correlation with disease states, pleural fluid granulocyte counts, and total pleural fluid white blood cell counts. The relationship between pleural fluid and blood lysozymes appears similar for both the transudates and exudates. The indirect evidence from this study suggests that pleural fluid lysozyme may be derived primarily from the blood rather than the product of inflammatory or neoplastic cells in the pleural fluid. This may be explained on the basis of the low molecular weight of lysozyme (14,000–15,000) and its readiness to diffuse into the pleural space from the circulation. Previous studies have demonstrated a lack of correlation of tear (14) and synovial fluid (9) with plasma lysozyme. This may be the result of active transport, production, or release of the enzyme by lacrymal gland or inflamed synovium. There was no evidence of a similar mechanism involved in the determination of pleural fluid lysozyme activity.

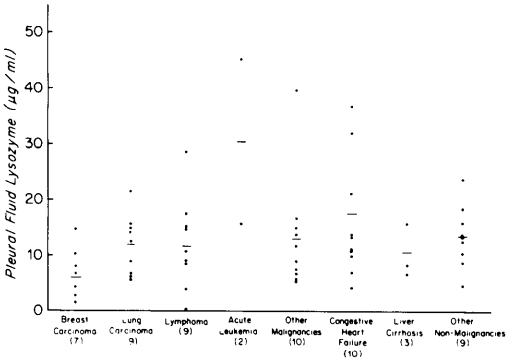

FIG. 1. Pleural fluid lysozyme in malignant and nonmalignant disorders. Parentheses enclose the number of patients in each group.

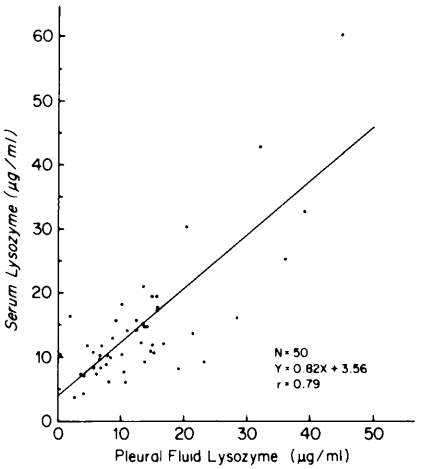

FIG. 2. Lysozyme activity in paired serum and pleural fluid samples.

Summary. A prospective study was conducted to define the content, significance, and source of lysozyme present in the pleural fluid in human diseases. The pleural fluid lysozyme activity is similar in various malignant and nonmalignant transudates and exudates, and is of limited diagnostic value. The pleural fluid activity correlated well with that of paired serum samples but it had poor correlation with the disease state, the pleural fluid granulocyte counts, and total white blood cell counts. The data suggest that the pleural fluid lysozyme may be derived primarily from the blood and that it

is not the product of inflammatory or neoplastic cells in the pleural fluid itself.

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