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Pathology

Study of Pleural Effusion Cytology in Neoplastic and Non-Neoplastic Conditions in Correlation with Adenosine Deaminase Levels

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Abstract **Original Research Article**

Introduction: The term serous effusion refers to the fluid collected in the three serous cavities namely pleural, peritoneal and pericardial. Serous effusions form an important source of useful diagnostic information in clinical practice. Pleural effusion cytology is the simplest definitive method available to diagnose the disease, which are the causes of pleural effusion. Diagnostic cytology depends on the fact that cells exfoliated or collected from a tissue surface reflect changes occurring in the underlying tissue. ***Materials & Methods:*** This study was conducted for a period of two years. The study was prospective based on cases admitted as in-patients. Total 235 cases were taken, and the ages of patients ranged from 14 years to 70 years and the group consisted of 170 male patients and 65 female patients. ***Results:*** Out of the total 235 patients with pleural effusion 196 were non-malignant and 39 were malignant. Out of the 235 effusion samples the majority were of non-specific compromising 47%. Next common was specific inflammation effusion comprising 33% next was malignant effusion comprising 17%. The unconfirmed effusion 3% were at the end of work up remained in conclusive. ***Conclusion:*** The present study demonstrated that the most useful single test in establishing the diagnosis of pleural effusion is the pleural fluid cytological study (60%). In conclusion, an ADA level of pleural fluid is a non-invasive, inexpensive and repeatable test that provides the results quickly. This study indicates that the ADA levels of the pleural fluid can be used with high diagnostic rates in the diagnosis and exclusion of the tuberculosis in patients whom the pleural tissue could not be obtained with various causes in the differential diagnosis of Tuberculosis and Malignant effusions, and those with waiting for the laboratory outcomes of the pleural tissue. In non-biopsy based Tuberculosis diagnosis a threshold of 47 IU/dl is most useful.

Keywords: Pleural effusion, adenosine deaminase, neoplastic, non neoplastic.

INTRODUCTION

With a simple bedside procedure thoracentesis, the fluid can be rapidly sampled and observed, its constituents examined microscopically and its contents qualified, a comprehensive and systemic approach to analysis of fluids in conjugation with knowledge of relevant clinical features and radiological findings can establish the diagnosis definitively viz. malignancy, empyema, tuberculous pleurisy, fungal infection of pleural space, lupus nephritis, chylothorax, urinothorax, esophageal hemothorax, peritoneal dialysis, extra vascular migration of venous catheter. However even with the non-diagnostic thoracentesis pleural effusion analysis will be useful in excluding other possible causes of pleural effusion.

The determination of the Adenosine deaminase (ADA) in the suspected pleural fluid appears to be the most promising marker because of the ease, rapidity and cost effectiveness of the ADA assay. ADA is a polymorphic enzyme involved in purine metabolism, it catalyzes the deamination of adenosine to the inosine and ammonia, although found in most tissues ADA activity is greatest in the lymphoid tissue. Its activity being 10 to 20 times more in T lymphocytes than in B lymphocytes, especially in the proliferation and differentiation of T lymphocytes. For that reason ADA has been looked on as a marker of cell mediated immunity, which encompasses the delayed hypersensitivity reaction [1].

Several studies have shown diagnostic utility of ADA in pleural effusion, it plays a role in the maturation of monocytes to macrophages in pleural fluid, it reflects the cellular immune response.

There are several isoforms of ADA, but the prominent ones are ADA_1 and ADA_2 which are coded by different gene loci. ADA_1 isoenzyme is found in all

cells with the highest concentration in lymphocytes and monocytes, whereas ADA_2 isoenzyme found only in monocytes. ADA_2 is the more efficient marker of tuberculous pleural effusion. Thus ADA levels can be used to effectively differentiate a malignant process from an infective process.

MATERIALS & METHODS

This study was conducted for a period of two years. The study was prospective based on cases admitted as in-patients. Total 235 cases were taken, and the ages of patients ranged from 14 years to 70 years and the group consisted of 170 male patients and 65 female patients.

The cause of pleural effusion was worked up in the following order upon admission

HISTORY

Clinical Examination

Laboratory Investigations

Under local anesthesia with strict aseptic precautions using a 3-way cannula pleural fluid was tapped into a clean, sterile dry container and taken to laboratory for processing as soon as possible. If any delay is anticipated it was refrigerated at 4^0C but not frozen.

The fluid was stirred and split into three containers and sent to Biochemistry, Microbiology and Pathology laboratories respectively.

The fluid received in the pathology laboratory was evaluated in the following way:

When the specimen is received in the laboratory, the gross appearance and the amount of fluid received is noted. The fluid may be clear, transparent, straw coloured, yellow, brown, red, chylous, purulent, mucoid or hemorrhagic.

- In effusions due to infective process, the fluid is likely to be cloudy and a Gram stain may immediately yield a diagnosis.
- The presence of Chylous fluid can be related to obstruction or injury to large lymphatic channels. Because of the lymphatic obstruction induced, extensive intra-abdominal lymphomas have been noted to present with chylous effusion. Conversely, a relatively common cause of chylous ascites in patients with known malignancy is a damage of lymphatic after external beam radiation.

A representative amount of fluid is taken after brisk shaking of the container and the procedure performed on improved Neuerbauer chamber either undiluted or in very small dilution

Differential Count

A) Chamber differential- Here in we divide the nucleated cells into polymorpho nuclear cells and Mono nuclear cells and 100 cells are counted. The nuclear stain can often be seen because of the small amounts of stain in the diluting fluid.

B) Smear method- Here in air dried smears are stained by Leishman Stain and the cells are counted.

The fluid as soon as it is received is stirred briskly, this causes the cells suspended in it to be dispersed and it prevents coagulation. A representative amount of the fluid (20ml-30ml) is centrifuged for 10 minutes at 2000 r.p.m. If the quantity of the fluid obtained on aspiration is too little for centrifugation, it is mixed with an equal amount of normal saline before centrifugation. If fibrin has already formed, small fragments are removed with the help of applicator sticks.

The supernatant part is discarded. A thin tightly wound cotton swab is dipped in the sediment button/buffy coat of the sediment and gently rolled over the surface of the slide. It is important that the smears are thin so that the cells lie in a monolayer. Two smears are made for each specimen and extra air-dried smears should be prepared and kept to be used later in case there is need for special staining to be done.

A wide variety of staining techniques are available for cytological preparations. As with any other technique, individual preference and experience may determine the choice of a particular method. However routine use of the standard Papanicolaou technique yields consistently satisfactory results in the field of diagnostic cytology.

RESULTS

The study population involved a total of 235 patients with pleural effusion. There were 170 male patients and 65 female patients. Age group ranged from 14 to 70 years with a mean age of 42 years. All patients were diagnosed clinically and confirmed by Chest X-ray/ Ultra sonogram, Diagnostic thoracentesis and occasionally closed tube thoracotomy were done with collected samples sent to the laboratory for analysis.

Gross fluid analysis ranged from clear yellow to grossly bloody.

Out of the total 235 patients with pleural effusion 196 were non-malignant and 39 were malignant. Out of the 235 effusion samples the majority were of non-specific compromising 47%. Next common was specific inflammation effusion comprising 33% next was malignant effusion comprising 17%. The unconfirmed effusion 3% were at the end of work up remained in conclusive.

Total number of cases affecting males were 170 (75%) and females 65 (25%). Male Female ratio = 3:1. The age of the patients ranged from 14 years to 70 years with the mean age of 42 years.

Of the total non-specific inflammatory effusions 110 (47%) further work up led to the diagnosis of the following subsets (Table-1).

Table-1: Showing various non specific inflammatory effusions

Post pneumonic	47
Congestive cardiac failure	27
Liver cirrhosis	12
Chronic renal failure	10
Pulmonary infarct	5
Unknown cases	9

Of the specific inflammatory effusion 77 (33%) the following lesions were noted (Table-2).

Table-2: Showing various specific inflammatory effusions

Tuberculosis	75
Fungal infection	2

Of the total malignant effusions 39 (17%) further work up revealed the following subsets (Table-3).

Table-3: Showing various malignant effusions

Bronchial carcinoma	23
Metastatic carcinoma	9
Lymphomas	2
Others	5

Out of the 235 cases submitted to the Department of Biochemistry the following results were obtained.

In those cases which had tuberculosis confirmed by various other parameters the mean serum ADA level was 15.32 ± 6.14 and the corresponding pleural effusion sample ADA level was 52.61 ± 26.9. In those cases which had malignant effusion the mean serum ADA level was 12.25± 12.2 and the corresponding pleural effusion sample ADA level was 23.14 ±11.14.

The mean pleural fluid (PF) and Serum (S) Ratio for ADA level in tuberculous patients was 3.78± 2.58 U/L. The mean pleural fluid (PF) and Serum (S) ratio for ADA level in those patients which had malignancy was 1.90 ± 1.17 U/L.

DISCUSSION

During 2 years study period we evaluated 235 patients with pleural effusion. The clinical recognition of a pleural effusion signifies that an abnormal physiologic state exists whereby there is disequilibrium in the formation and removal of pleural fluid. The pleural fluid commonly is the sequel of primary pulmonary diseases, but can also result from diseases in extra pulmonic focus such as heart (congestive cardiac failure), kidneys (Nephrotic syndrome), Liver (cirrhosis) and the Pancreas (Acute pancreatitis). Pleural effusion can also can occur with systemic diseases (SLE, Yellow nail syndrome), metastatic malignancy and Iatrogenic causes such as drug therapy (Nitrofurantoin) and extravascular migration of central venous catheter.

As quoted by Light *et al*., 1973 Osler reported that 95% of blood tinged pleural effusions were malignant. In our study 80% of malignant pleural effusion were hemorrhagic. Light *et al.*, in their study have shown that prominent inflammatory cells associated with malignant effusions were the lymphocytes mainly followed by monocytes and neutrophils. Our study showed a similar observation [2].

Cell count and differential count did not contribute in any confirmative manner in any diagnosis. Cytologic examination of body fluids is of distinct value in confirming or disapproving malignant metastatic tumors to the cavities.

This method is of more of value in prognosis Foot N. C 1956 because the findings of cancer cells in such a specimen denotes that the patient has cancer that is not only advanced but also almost always incurable.

The cells were scanty especially in the cases of transudates, however the morphology of cells were well preserved even a few ml of effusion fluid was sufficient to make smears and to give good information as in the present study. In our experience even though specimens stored at 4^0C overnight showed that cells retained their morphology to a considerable extent. Howsoever fresh samples offered distinct advantages.

In our study, the initial thoracentesis analysis showed an average of 58% positive cytology, and a repeat thoracentesis increased the diagnostic yield to 68% 72% in Barter *et al.*, 1994 and a third thoracentesis increased the cytological yield to 72% (77% in Barter *et al.*, 1994) [3].

The fluid act as a good culture medium and the exfoliated cells benign or malignant will continue to divide in these fluids, which explains why cells in mitosis are more likely to be seen in effusions than in any other types of tissues Naib Z N 1970 [4]. Multinucleation and Mitosis were seen in 90% of our cases.

One should not give undue importance to mitosis in effusion fluids and over diagnose

malignancy. The diagnosis of malignancy solely rest on nuclear features in effusions.

Mesothelial cells in fluids varies from one disease to another, in tuberculosis they are scanty due to covering of the pleural surfaces [4-6]. However this cytological evidence of tuberculosis have to be analyzed in the light of Pleural effusion ADA which is invariably increased, with biopsy and AFB stain.

In as many as 20% of all pleural effusions, the basic investigations done and said in the beginning did not establish the diagnosis and even a Thoracotomy or Thoracoscopy may not reveal the cause of effusion.

The fluid in tuberculosis collects as a result of a delayed hypersensitivity reaction to tuberculous proteins. The numbers of bacteria are few, and smears and culture has a low diagnostic yield (<25%). However, most tuberculous effusions are exudates and have a low glucose concentration, although the later does not discriminate tuberculous from malignancy, parapneumonic effusions or Rheumatoid diseases.

Adenosine De Aminase (ADA) assay is a cheap, simple, diagnostic test for tuberculous pleurisy (Thorax 1980). Investigation of a pleural effusion demands a pleural aspiration and biopsy.

Countries with a high prevalence of tuberculous pleural effusions have a high degree of specificity and sensitivity for the ADA test, which makes it an integral part of a diagnostic work up of lymphocyte rich exudative body fluids. It should be added to the armamentarium of the diagnostic work up of body fluids in patients who are suspected of having tuberculosis.

Histopathological and Microbiologocal analysis of pleural fluid or tissue may seem as the most ideal method, but in 20% of patients definitive diagnosis cannot be reached [7, 8]. Hence many markers that may be helpful in the differential diagnosis were studied in the pleural fluid. Two of these, ADA and Interferon gamma are most widely used and currently the most accepted tests.

But ADA has been more commonly preferred for the diagnostic algorithms in the countries with a moderate to high incidence of tuberculosis because it is more inexpensive method that can be accessed more quickly.

CONCLUSION

Cytological examination of fluids obtained from the serous cavities is among the most common tasks performed in the practice of cytopathology. The common reason to submit an effusion to cytopathology is to determine whether or not it contains malignant cells. This study which was carried out in 235 patients who had varying quantities of pleural fluid accumulation with or without parenchymal lesions were subjected to the a fore mentioned investigations.

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