CLINICOBIOCHEMICAL CORRELATION AND CYTOLOGICAL STUDY OF PLEURAL FLUID

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***Abstract*: Cytological examination of fluids is of great diagnostic value in both non-neoplastic and neoplastic effusions. Apart from reporting malignancies, cytological evaluation also reveals information about various inflammatory conditions of serous membranes, fungal, bacterial, parasitic and viral infections. Methods: A prospective study carried out at a tertiary care hospital in Navi Mumbai over a period of 2yrs. A total number of 100 cases were studied. They were examined for physical appearance, protein and ADA levels. The pleural fluid was examined for cytomorphological features by using routine and special stains. Results: Out of the 100 cases, 4 cases were clinically diagnosed to be malignant, out of which 2 cases were positive for malignancy on cytological examination. Taking total protein cut off value of 3gm/dl, 65 fluids were exudative and 35 were transudative in nature. 88.2% of the cases diagnosed as tuberculosis with pleural effusion showed raised levels of ADA.A cut off value of 40 U/L of ADA for pleural fluids showed a sensitivity of 88.2%, specificity of 70.1% and a PPV and NPV of 46.8% and 95.2% respectively. Conclusion: Though the cytological examination of the fluids seems to be simple, yet its diagnostic importance is of great significance particularly in cases of malignancy, tuberculosis and peritonitis.**

***Keywords*: Body fluid, Cytology, Malignancy**

I. INTRODUCTION

Effusion means abnormal accumulation of fluid within the body cavities. Evaluation of cytological examination of body fluids is of great diagnostic value in both non-neoplastic and neoplastic effusion and it is a rapid, simple, cost-effective. Since cytological examination is both diagnostic as well as therapeutic intervention, tapping of these body fluids helps in better understanding of the disease process.[1] Most commonly analysed fluids are pleural, ascitic, pericardial, synovial and occasionally peritoneal fluid/wash. Some of the causes of pleural and peritoneal effusions include congestive cardiac failure, cirrhosis, neoplasms and infections. Pleural effusions and ascitic fluids are classically divided into transudates and exudates and this is the first step as it often provides an indication of the underlying pathophysiological process along with the differential diagnosis and thus further investigation can be suggested.[2] Transudative fluids are clear with low protein levels less than 3gm% and glucose levels similar to serum levels. Exudative fluids are slightly hazy fluids with high protein levels more than 3gm% and low glucose levels.[3] Exudative fluids can be further investigated by cytopathology for definite diagnosis whereas transudative fluids should be treated for the underlying cause. Nearly all malignancies can commonly present with or develop pleural or peritoneal effusion. The differentiation of the fluid into malignant or non-malignant fluid has a deep impact on the course of treatment to be followed. Though, the tumors often shed abundant malignant cells, singly and in clusters. The diagnosis of malignancy is much difficult in any type of body fluid because of the rapid proliferation of cells within the fluids.[4] Effusions are usually the first clinical symptom in malignant tumors or of their metastatic manifestation. Cytological examination is considered as the first, best or only chance for making the diagnosis of an underlying malignancy. Negative for malignant cells does not exclude the presence of malignant neoplasm and at times such patients may present with difficult diagnostic problems. The present study is undertaken to differentiate ascitic and pleural fluids into transudates and exudates by using fluid protein levels, to detect malignancy, find the significance of ADA levels, especially in tuberculosis and also to correlate with clinical diagnosis.

The **aims and objectives** of the study is to find clinicopathological correlation of pleural fluids, to correlate with biochemical parameters, to detect malignancy in suspected cases on cytological stains and to study the importance of ADA in specific cases.

II. MATERIALS AND METHODS

Study Area: The present study was conducted in the dept. of Pathology, in a tertiary care hospital in Navi Mumbai.
Study Population: Total numbers of 100 cases were included in this study.
Study Duration: The duration of study was over a period of two years.
Data collection: The study included all samples of pleural fluids received in pathology department. These fluids were analysed for physical properties like the volume, colour and viscosity. The fluids were analysed for biochemical parameters such as protein and ADA. Total WBC and RBC counts of fluid were carried out using Neubauer's chamber. Then fluids were centrifuged for 15minutes at 1500 rpm. From the sediment smears were prepared and stained by Field's and Leishman's stain for differential count and Papanicolaou stain for cytological study.

III. RESULTS

Out of 100 pleural fluids received in pathology central laboratory, 96 cases were diagnosed as benign aetiologies clinically and 4 cases were diagnosed as malignant lesions.

Table 1: Age distribution of different pathologies in 100 cases of pleural effusion

DIAGNOSIS	AGE IN YEARS					
	<10 YEARS	10-20 YRS	21-30 YRS	31-40 YRS	41-50 YRS	>50 YEARS
PNEUMONIA	15	03	07	01	02	04
TUBERCULOSIS	02	04	01	04	08	02
HYPOPROTEINEMIA	-	-	-	04	02	06
CKD	-	-	-	-	07	03
CCF	-	-	-	02	01	05
TRAUMA	-	-	01	03	01	01
NEPHROTIC SYNDROME	-	02	01	-	01	-
MALIGNANCY	-	-	-	01	-	03
LIVER DISEASE	-	-	-	01	02	-

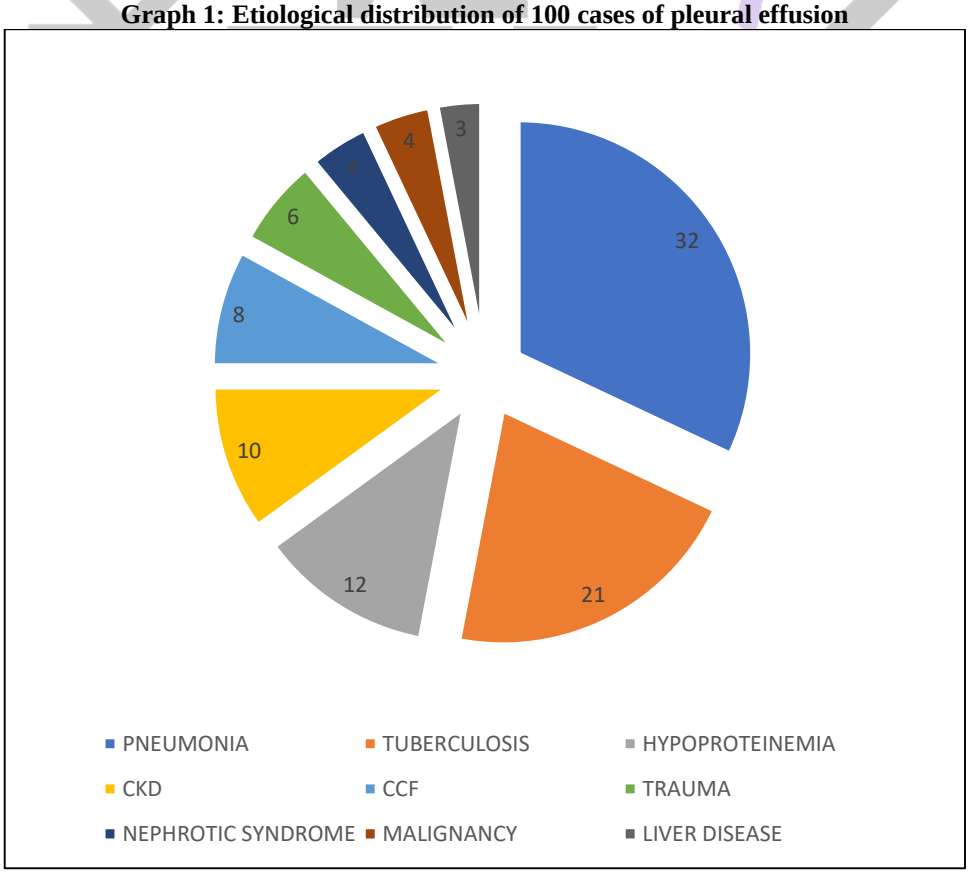
Predominant age group of patients with pneumonia was <10yrs and with tuberculosis was 41-50 yrs.

Table 2: Sex-wise distribution of different pathologies in relation to pleural effusion cases

DIAGNOSIS	FEMALE	MALE
PNEUMONIA	**12**	**20**
TUBERCULOSIS	05	16
HYPOPROTEINEMIA	08	04
CKD	04	06
CCF	05	03
TRAUMA	01	05
NEPHROTIC SYNDROME	03	01
MALIGNANCY	03	01
LIVER DISEASE	-	03
TOTAL	**41**	**59**

It was observed that there was a slight male predominance where 59% of the patients with pleural effusion were males and 41% were females.

Graph 1: Etiological distribution of 100 cases of pleural effusion



Pneumonia was found to be the most common cause (32%) followed by tuberculosis (21%). Only 3% of the pleural effusions were seen in liver diseases.

Table 3: Total Fluid protein levels in 100 cases of pleural effusion

DIAGNOSIS	TOTAL PROTEIN (gm/dl)	
	>3	<3
PNEUMONIA	25	07
TUBERCULOSIS	16	05
HYPOPROTEINEMIA	07	05
CKD	06	04
CCF	03	05
TRAUMA	02	04
NEPHROTIC SYNDROME	-	04
MALIGNANCY	03	01
LIVER DISEASE	03	-
TOTAL		

65% were exudative in nature, maximum cases of pneumonia followed by tuberculosis and 35% of the fluids were transudative in nature.

Table 4: ADA levels in 74 cases of Pleural Effusion

DIAGNOSIS	ADA > 40	ADA < 40
PNEUMONIA	09	10
TUBERCULOSIS	15	02
HYPOPROTEINEMIA	01	10
CKD	01	07
CCF	02	03
TRAUMA	01	04
NEPHROTIC SYNDROME	-	02
MALIGNANCY	02	02
LIVER DISEASE	01	02
TOTAL	**32**	**42**

Fifteen cases (88.24%) of tuberculosis showed raised ADA level in pleural fluid.

Table 5: Total leucocyte count in 100 pleural fluids

DIAGNOSIS	TOTAL LEUCOCYTE COUNT IN CELLS/mm^3		
	<1000	1000-5000	>5000
PNEUMONIA	07	15	10
TUBERCULOSIS	07	07	07
HYPOPROTEINEMIA	03	07	02
CKD	06	03	01
CCF	03	05	-
TRAUMA	04	02	-
NEPHROTIC SYNDROME	02	01	01
MALIGNANCY	01	02	01
LIVER DISEASE	01	01	01
TOTAL	**34**	**43**	**23**

78% of cases of pneumonia and 67% of cases of tuberculosis showed WBC count >1000cells/mm^3 in the pleural fluid.

Table 6: Gross appearance of 100 cases of pleural fluid with etiology

DIAGNOSIS	CLEAR	SLIGHTLY HAZY	HAZY	TURBID
PNEUMONIA	01	11	18	02
TUBERCULOSIS	03	08	09	01
HYPOPROTEINEMIA	02	07	03	-
CKD	04	04	02	-
CCF	01	05	02	-
TRAUMA	02	02	02	-
NEPHROTIC SYNDROME	-	-	04	-
MALIGNANCY	-	-	04	-
LIVER DISEASE	-	01	02	-
TOTAL	**13**	**38**	**46**	**03**

67% of pleural fluids which appeared turbid were caused due to pneumonia and the rest 33% were due to tuberculosis. Also, only 1(3%) pleural fluid caused by pneumonia was clear.

Table 7: Gross examination of color of 100cases of pleural fluid with etiology

DIAGNOSIS	PALE YELLOW	STRAW	RED	COLOURLESS	WHITISH
PNEUMONIA	15	09	08	-	-
TUBERCULOSIS	18	01	01	-	01
HYPOPROTEINEMIA	08	02	01	-	01
CKD	09	-	-	01	-
CCF	07	-	01	-	-
TRAUMA	04	-	02	-	-
NEPHROTIC SYNDROME	01	-	02	-	01
MALIGNANCY	-	01	03	-	-
LIVER DISEASE	03	-	-	-	-
TOTAL	**65**	**13**	**18**	**01**	**03**

18(85.7%) of the pleural fluids due to tuberculosis were pale yellow in color. 3(75%) of the pleural fluids due to malignancy were red in color.

Table 8: Clinicocytological correlation in cases of malignancy presenting with effusion

NATURE OF SPECIMEN	NO. OF CASES CLINICALLY DIAGNOSED AS MALIGNANCY	CYTOLOGICALLY POSITIVE FOR MALIGNANCY	PERCENTAGE
PLEURAL FLUID	04	02	50%

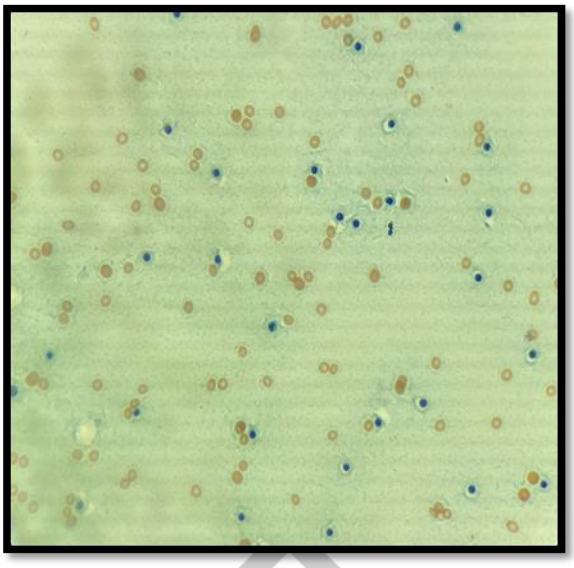


Figure 1: Lymphocytic Effusion (40x)

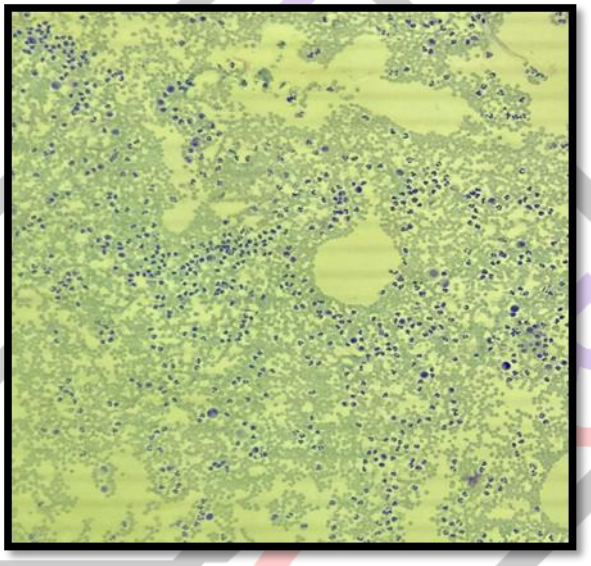


Figure 2: Purulent Effusion (10x)

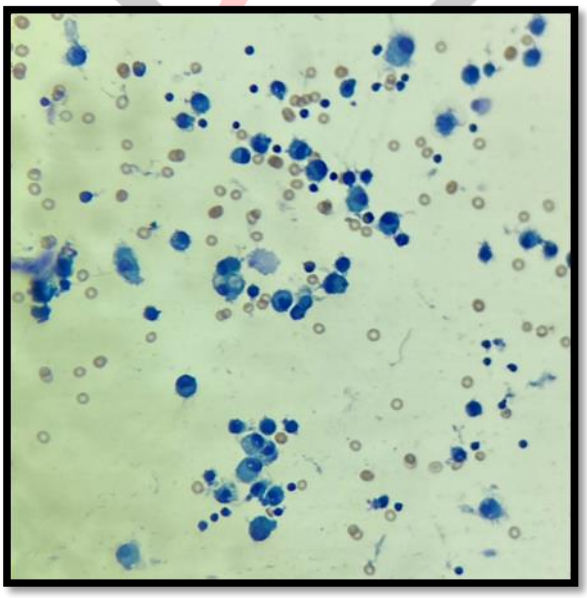


Figure 3: Mesothelial cells and Macrophages (40x)

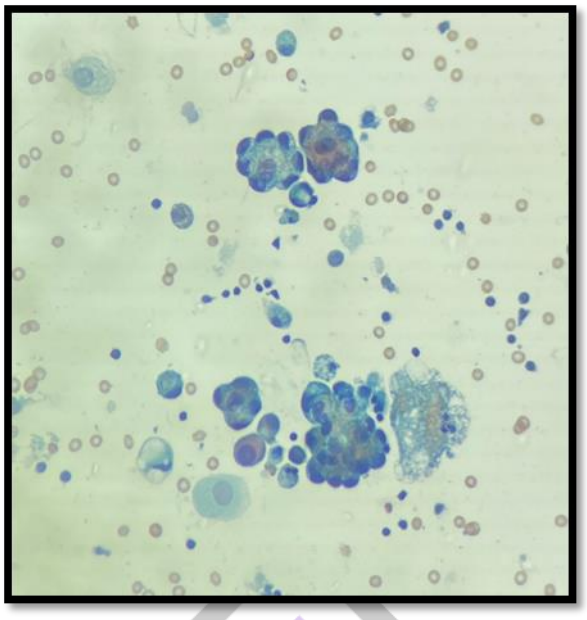


Figure 4: Malignant Effusion (40x)

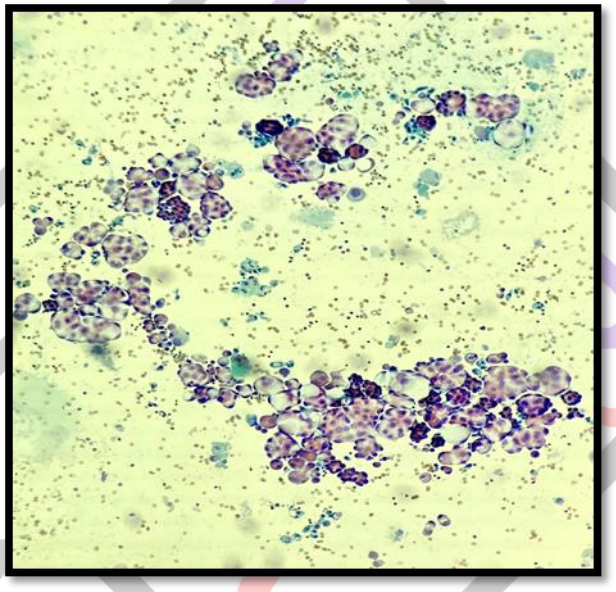


Figure 5: Malignant Effusion [Mesothelioma in Pleural Fluid] (10x)

IV. DISCUSSION

The pioneers of effusion cytology were Lucke and Kiebs (1867). Malignancy in pleural effusion was first described by Quincke in 1882.[1] Over the years different pathologies have come across in the literature, which are potential aetiologies for effusions. Owing to these facts the exact diagnosis of the underlying disease is known. It has gained increased acceptance in clinical practice today, since body fluid aspiration for cytological and biochemical study is relatively simple, safe and inexpensive procedure.[5] Thus, the number of samples received in pathology laboratory is increasing and the clinicians use the effusion cytology report to diagnose and treat the underlying cause. In addition to cytological evaluation, clinical correlation and biochemical analysis of these fluids is also important.

Pleural effusion represents a very common diagnostic problem. It occurs in a variety of diseases. Amongst the various etiologies, exudative pleural effusion is caused by tuberculosis, malignancy and synpneumonic effusion while transudative pleural effusion is commonly associated with anaemia- hypoproteinemia and congestive cardiac failure. In the present study, 65% fluids were exudative and remaining 35% of the fluids were transudative. These findings were in concordance with Light et al [6] and Joseph et al study.[7] In the present study synpneumonic effusion (32%) was found to be the most common cause of pleural effusion which was in concordance with findings of Romero et al.[8] The incidence of malignancy in present study was 4% which is comparatively lower than the findings of Hirsch A et al [9], Light et al.[6] The male population (59%) is more affected than the females (41%) in our study, which was in concordance with other studies.[7,9,10] In our study 25% males and 755 females were affected in 4% of the total cases which were malignant and majority of them (75%) were beyond 5th decade. On cytological examination, in cases of tuberculosis, equal number of patients showed total WBC count > 1000 cells/cumm, between 1000-5000 cellls/cumm and <1000 cells/cumm. In malignancy >1000 cells/cumm were observed in 75% of the cases. In synpneumonic effusion, 78.12% cases showed >1000 cells/cumm and remaining cases showed <1000 cells/cumm. All these findings were in concordance with the findings of Light et al.[6] in the present study, lymphocytic predominance was found in 85.7% of the cases of tuberculosis and in all cases of malignancy

which is in accordance with Bagahna M.F et al[11]. On gross examination of pleural fluid, in tuberculosis the samples of pleural fluid were mostly pale yellow (75%) in the study which is similar to the reports of Bagahna M.F et al.[11] In malignant effusions, all the cases showed haemorrhagic fluid and were hazy, which is similar to findings of Leuallen EC et al[12] and Light et al.[6] 88.2% of the cases diagnosed as tuberculosis with pleural effusion showed raised levels of ADA. A cut off value of 40 U/L of ADA for pleural fluids showed a sensitivity of 88.2%, specificity of 70.1% and a PPV and NPV of 46.8% and 95.2% respectively.

V. CONCLUSION

Biochemical studies along with cytological analysis of pleural fluid help in understanding the disease diagnosis. Since this is a simple, minimally invasive, less time consuming, cost effective and considered as a first line method in arriving at the diagnosis. This thereby reduces the need for invasive investigations and save the patient from the complications of invasive procedures. Cytological study of body fluids is also useful in finding the cause of effusion, in understanding the course of disease, in evaluating and staging malignancies thereby helps the clinician in further deciding the course of management. This results in staging of tumor and thereby affects treatment plan and prognosis for the patient.

VI. DISCLOSURE OF CONFLICT OF INTEREST:

The authors have no potential conflicts of interest to disclosure.

VII.SOURCE OF FUNDING:

No funding was needed.

VIII.ETHICAL APPROVAL:

The study was approved by the Institutional Ethics Committee.

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