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Total Protein estimation and differential leukocyte count of pleural fluid in tuberculosis.

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Abstract: Alteration of the permeability of the pleural surface, so that the pleura is more permeable to fluid and larger molecular weight components of blood like proteins.The leading causes of exudative pleural effusions are bacterial pneumonia, malignancy, viral infection and pulmonary embolism.Tuberculosis is caused by bacteria belonging to the Mycobacterium tuberculosis complex. Tuberculosis can be diagnosed by analysis of plural fluid. **AIMS AND OBJECTIVES :** Study has been undertaken to evaluate the diagnostic significance of total protein estimation of pleural fluid along with its differential leukocyte count

Introduction

Pleural fluid is a clear fluid which is present as a very thin layer between the lung and the chest wall. It serves as a coupling system between the lung and the chest wall. Pleural fluid is present as a thin layer in the pleural space. Pleural fluid accumulates when pleural fluid formation exceeds pleural fluid absorption. Normally, fluid enters the pleural space from the capillaries in the parietal pleura and is removed via the lymphatics situated in the parietal pleura.

Fluid also can enter the pleural space from the interstitial spaces of the lung via the visceral pleura or from the peritoneal cavity via small holes in the diaphragm. The lymphatics have the capacity to absorb 20 times more fluid than is normally formed. Accordingly a pleural effusion may develop when there is excess pleural fluid formation (from the parietal pleura, the interstitial spaces of the lung, or the peritoneal cavity) or when there is decreased fluid removal by the lymphatics.

Pathogenesis of pleural effusion :-

Pleural effusion may be due to changes in the following two categories:

1. Alteration of the permeability of the pleural surface, so that the pleura is more permeable to fluid and larger molecular weight components of blood like proteins. Local factors cause such alteration resulting in exudative pleural effusion. The leading causes of exudative pleural effusions are bacterial pneumonia, malignancy, viral infection and pulmonary embolism.

2. Alteration in the driving pressure, encompassing a change in hydrostatic or colloid osmotic pressures of parietal or visceral pleura, without any change in pleural permeability. Such alterations are caused by systemic factors resulting in transudative

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pleural effusion, The leading causes of transudative pleural effusions are left ventricular failure, pulmonary embolism and cirrhosis.

The primary reason to make this differentiation is that additional procedures are indicated with exudative effusions to define the cause of the local disease.

Transudative and exudative pleural effusions are distinguished by measuring the lactate dehydrogenase (LDH) and protein levels in pleural fluid. Exudative pleural effusion meet at least one of the following criteria, whereas transudative pleural effusions meet none.

1. Pleural fluid protein / serum protein > 0.5 i.e.,pleural fluid protein > 3 gms % .
2. Pleural fluid LDH / serum LDH > 0.6
3. Pleural fluid LDH is more than two - thirds of normal upper limit for serum

Pleural Effusion In Tuberculosis:-

Tuberculosis is caused by bacteria belonging to the Mycobacterium tuberculosis complex. The disease usually affects the lung, although in upto one - third of the cases other organs are involved. If properly treated, tuberculosis caused by drug - susceptible strains is curable in virtually all cases. If untreated, the disease may be fatal within 5 years in more than half of cases. Transmission usually takes place through the airborne spread of droplet nuclei produced by patients with infectious pulmonary tuberculosis. **MATERIALS AND METHODS**:Pleural fluid samples were taken from tuberculous and non-tuberculous patients in the Department of Respiratory Medicine of B.Y.L. Nair Charitable Hospital. 22 samples were studied of which 17 were known tuberculous exudates and 5 were non-tuberculous transudates which were taken as controls. The samples were proven bacteriologically and microbiologically to be exudates of tuberculous origin. Estimation of Total proteins done by Folin - Lowry method and Differential Leukocyte Count :- by Leishman's stain. **Result**: Plural fluid protein content is significantaly incresed in patients samples (Mean 3.88 gm%) as compared to controls (mean 2.18 gm%) p value is <0.001. Pleural fluid lymphocytosis, observed particularly lymphocyte counts of 85 to 90% of the total cells, suggests tuberculous pleurisy. **Conclusion**:The importance of the protein content of the pleural effusion has been stressed for a long time. It can be concluded from the result obtained that lymphocyte count along with the protein estimation has more diagnostic value as far as tuberculosis exudate is concerned.

KEY Words: **Tuberculosis, Plural fluid proteins and differtial leukocytes**

Etiological Agent :

Mycobacteria belong to the family Mycobacteriaceae and the order Actinomycetales. Of the pathogenic species belonging to the M.tuberculosis complex, the most frequent and important agent is Mycobacterium bovis (the bovine tubercule bacillus, once an important cause of tuberculosis transmitted by unpasteurized milk) and *Mycobacterium africanum.*

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AIMS AND OBJECTIVES

Study has been undertaken to evaluate the diagnostic significance of total protein estimation of pleural fluid along with its differential leukocyte count.

MATERIALS AND METHODS

Estimation of Total proteins by Folin - Lowry method :-

Principle : The protein estimation carried out by Folin - Lowry method is about 100 times more sensitive when compared to the Biuret method . It is quite sensitive for the estimation of low concentration of proteins (about 10 - 200 mg) .

The Folin-Lowry method for protein estimation is based on colour production by two reactions:

1. Biuret reaction of peptide bonds with copper ions in alkali and

2. Reduction of the phosphomolybdic acid - phosphotungstic acid by both, the Cu - protein complex and tyrosine and tryptophan present in the protein, in an alkaline medium .

The combined effect of the above two reactions changes the initial golden yellow colour of the Folin - Lowry reagent to deep blue colour due to the formation of molybdenum blue and tungsten blue. Tyrosine and tryptophan present in protein produce colour in the absence of copper ions, but the rest of amino acids in the proteins give a colour only in the presence of copper ions. About 75 % of the colour is dependent on copper ions,

The blue colour developed is measured at 670 nm or using a red filter

For samples and controls :-

Sample were from patients with tuberculosis and control were pleural fuild samples taken from non-tubreculous patients.

Tube Reagents	Blank	Standard	Sample/Control
0.85% saline	1ml	-	-
Standard protein (1mg/ml)	-	10ml	-
Sample/Control	-	-	10ml
0.85% saline	-	0.99ml	0.99ml
Lowry's reagent	5ml	5ml	5ml
Incubate at room temperature for 10 mins.			
Folin Lowryreagent	0.5ml	0.5ml	0.5ml
Incubate at room. Temperature for 30 mins. Read at 670 nm.			

2 Differential Leukocyte Count :- [2] by Leishman's stain

MATERIALS : -

Pleural fluid samples were taken from tuberculous and non-tuberculous patients in the Department of Respiratory Medicine of B.Y.L. Nair Charitable Hospital. 22 samples were studied of which 17 were known tuberculous exudates and 5 were non-

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tuberculous transudates which were taken as controls. The samples were proven bacteriologically and microbiologically to be exudates of tuberculous origin.

RESULTS

Table showing the total protein levels in pleural fluid samples and controls.

	Samples	Controls
No. of Samples/control	17	5
Mean	3.88 gm%	2.18 gm%
Standard deviation	+0.759	+1.0826
Standard error	+0.184	+0.484
Mean standard error	+0.4335	
T	3.829	
P	<0.001	

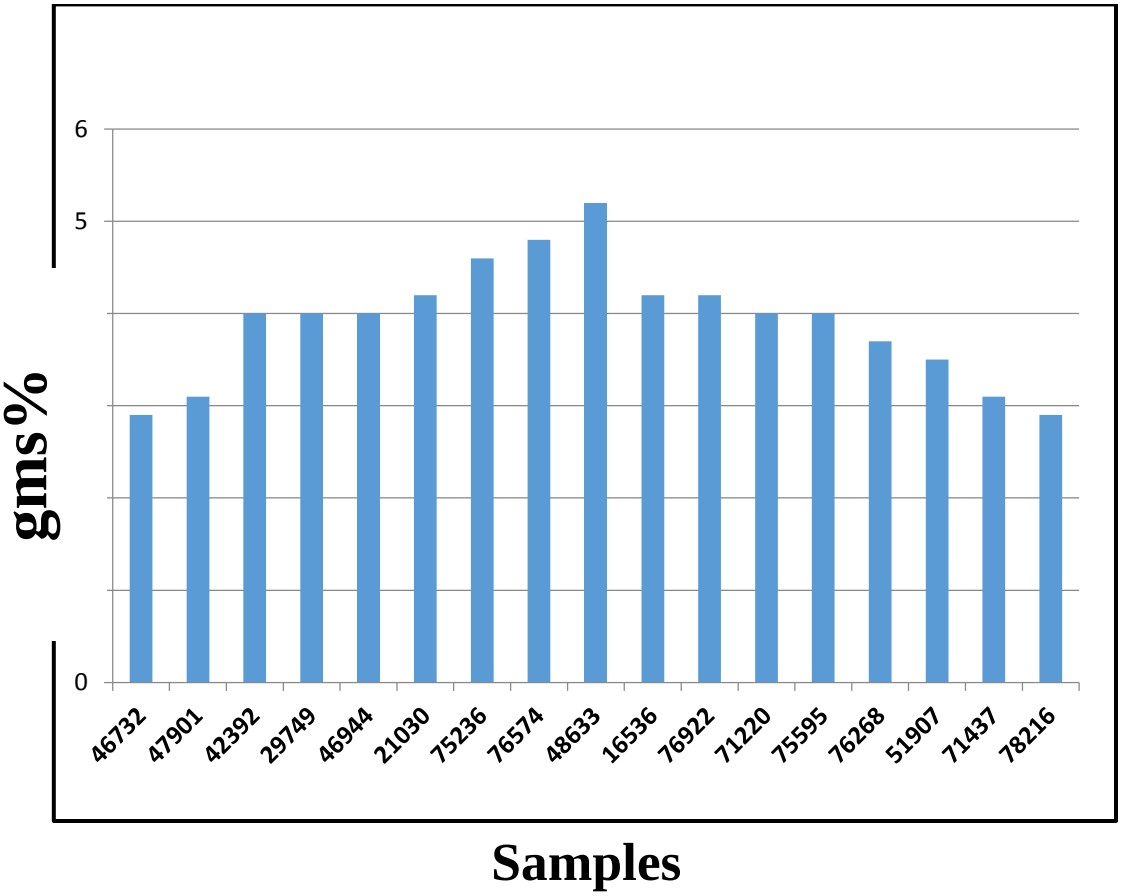

Diagram 1. Histogram showing total protein in pleural fluid samples

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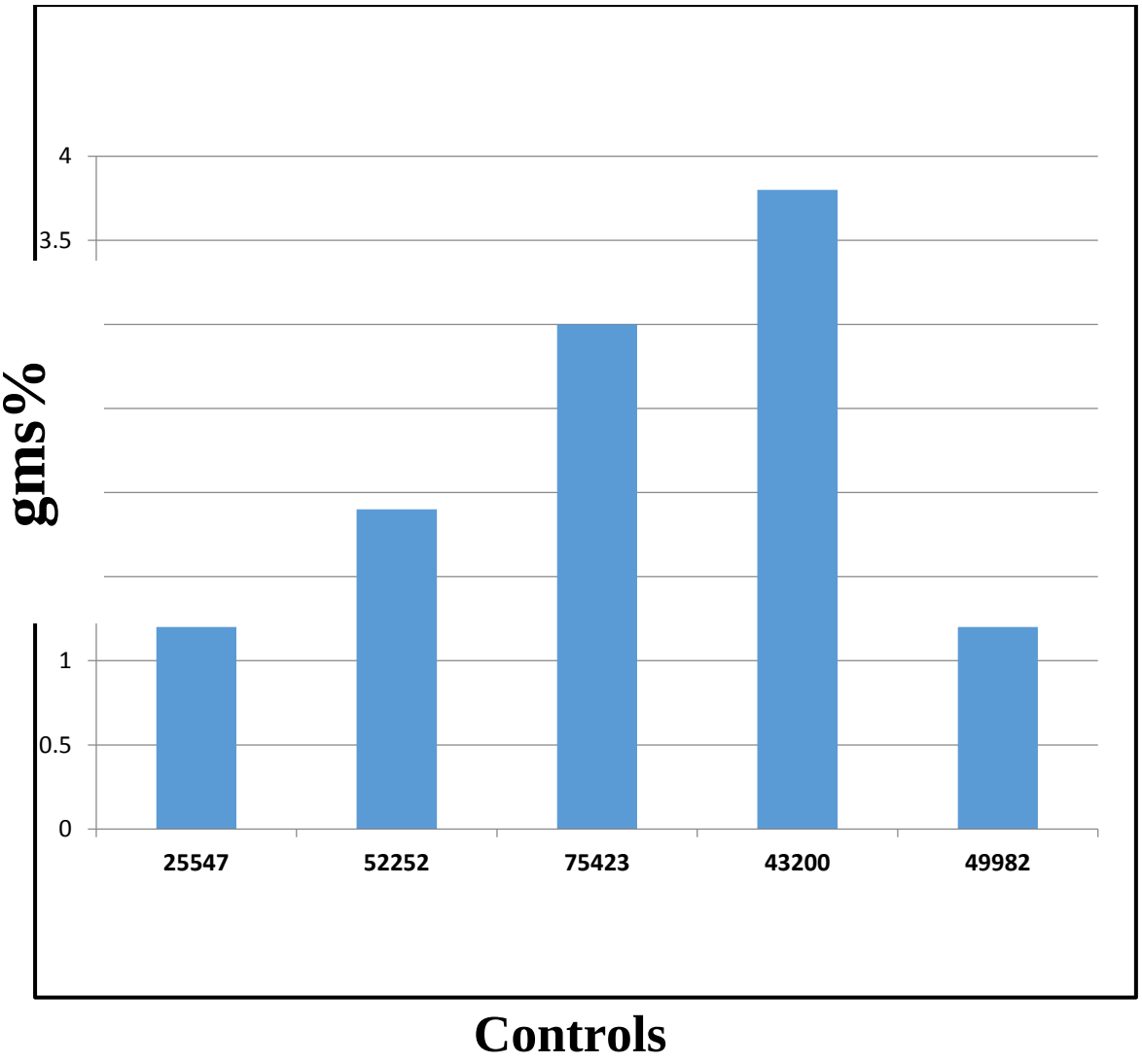

Diagram 2: Histogram showing total protein levels in pleural fluid controls

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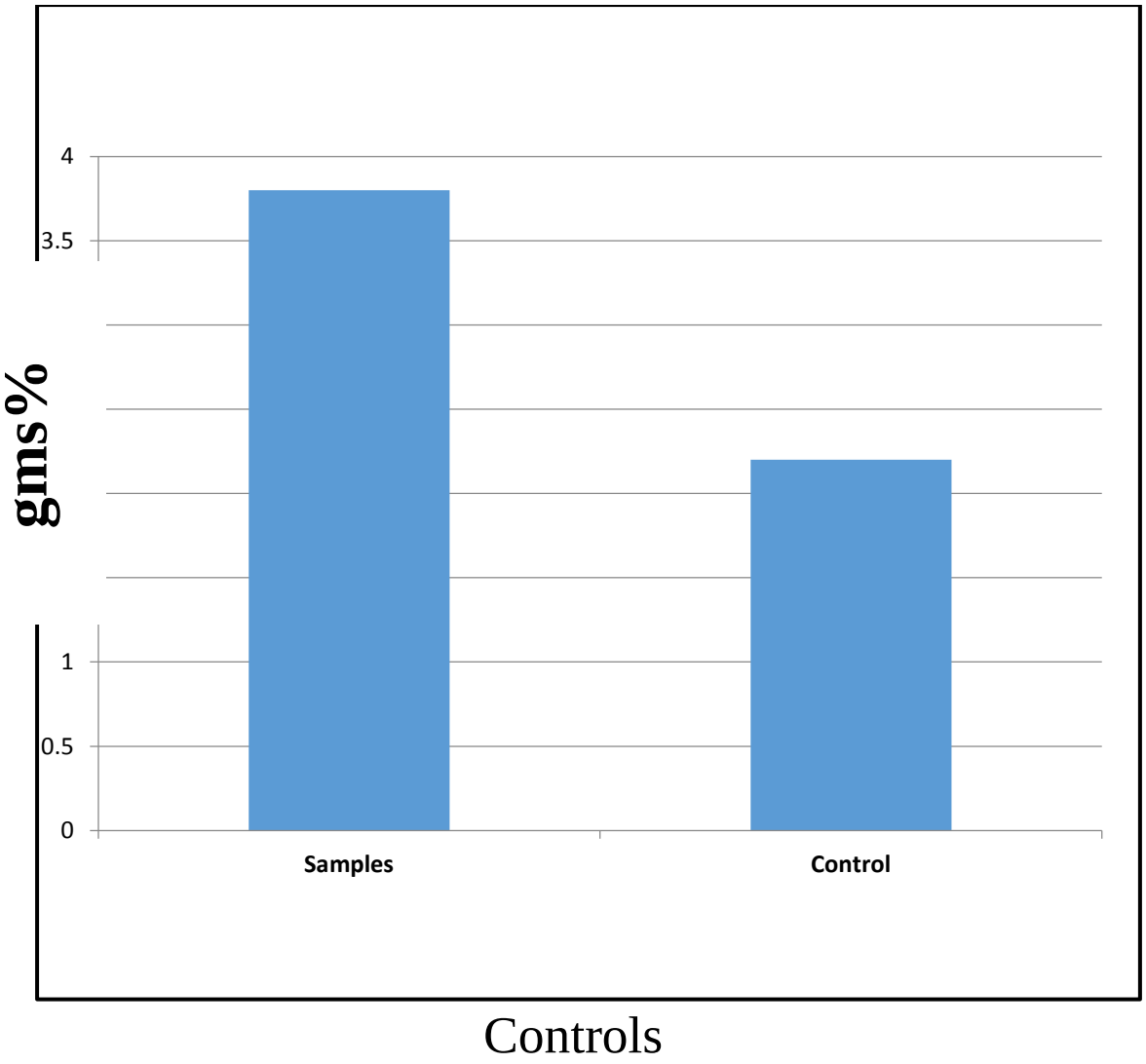

Diagram 3: Histogram showing the comparisons of the average total protein levels in pleural fluid samples and controls

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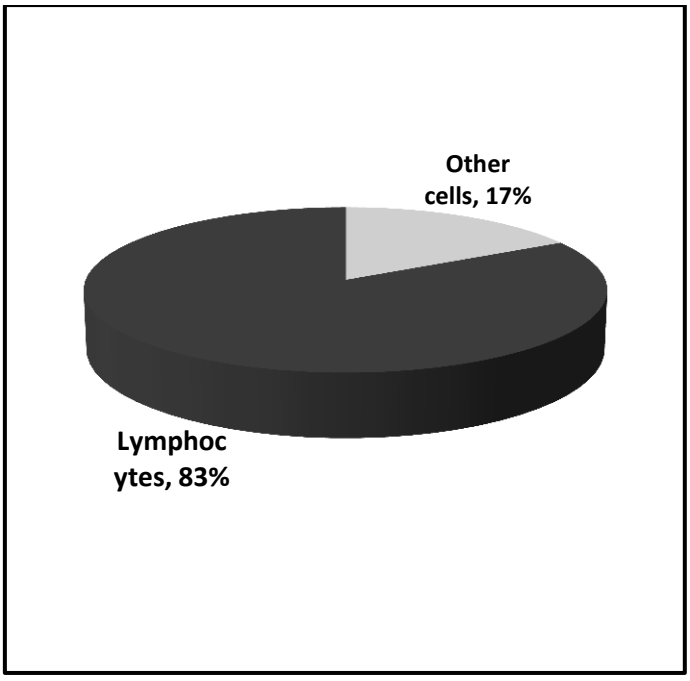

Diagram 4: Pie -chart showing the average percentage of lymphocytes present in pleural fluid samples.

DISCUSSION

Pleural effusion refers to the accumulation of excessive amounts of fluid in the pleural space. It is a frequent manifestation of serious pulmonary or cardiac disease and occasionally it is the first evidence of systemic disease. This effusions are often sub - divided into transudates, which are ultra filtrates of plasma having low concentration of protein and exudates which result from capillary damage or lymphatic blockage or infection and have high concentration of proteins.

The criteria used to define an exudate are : [3,4]

1. Pleural fluid - serum ratio of total protein of 0.5 or greater. [10,11]
2. Pleural fluid LDH of 20 IU or greater.
3. Pleural fluid-serum ratio of LDH of 0.6 or greater.

The simultaneous use of both the pleural fluid protein and LDH levels better differentiates transudates from exudates than does the use of one of these values individually.

When 17 samples of exudates of known tuberculosis cases are tested for their total protein content. The concentration of total protein were found higher than the normal range of pleural fluid total protein content (2-3 gm %) [13] These findings were expected as they were known tuberculous exudates .

Although cytological examination of effusions were recorded as early as in 1875, reports dealing with the significance of cytologic patterns other than neoplastic cells are few and sporadic.

It is difficult to define lymphocytosis of pleural fluid effusion since no normal data are available and all the effusions of clinical significance are pathologic. It is shown

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that the white blood cell count of pleural fluid would be helpful in separating exudates from transudates. Light et.al 5 have shown that more than 80% of transudates have white blood cell counts of less than 1000/mm whereas more than 80% of the exudates have white blood cell counts greater than 1000/mm They have also shown that transudates rarely had white blood cell counts greater than 1500/mm, but only 57 % of the exudates exceeded this value. Similar reports were also given by Paddock et.al.[6] Sahn .SA[7] has also shown that exudates in tuberculosis usually have less than 5000 leukocytes / mm but more than 1000 leukocytes/mm.

When the pleural fluid is grossly purulent the leukocyte count may be less than anticipated because the leukocytes have undergone lysis and the debris from the cells accounts for the turbidity - purulence of the fluid. Acute exudates are polymorphonuclear neutrophil predominant. As the time from the acute insult lengthens the effusion to mononuclear predominance if pleural injury is not persistent. Therefore in diseases in which the patients presents shortly following the onset of symptoms, such as bacterial pneumonia, pulmonary embolism and pancreatitis effusions, the PMN predominates.

Pleural fluid lymphocytosis, particularly lymphocyte counts of 85 to 90% of the total cells, suggests tuberculous pleurisy.5,8 However, lymphoma,8 sarcoidosis and chronic rheumatoid pleurisy need to be considered. Carinomatous pleural effusions will have > 50 % lymphocytes in two thirds of the cases.5,8 Since aforementioned diseases can be diagnosed by closed pleural biopsy, an undiagnosed exudate with lymphocytosis is the most appropriate indication for pleural fluid biopsy.

Pleural fluid eosinophilia12 suggest benign, self - limited diagnosis commonly associated with air or blood in pleural space.

Mesothelial cells are predominant in transudates and are found to a variable degree in exudates. The significance of mesothelial cells in exudates are that more than 5% virtually excludes the diagnosis of tuberculous pleurisy. [14]

The pleural fluid macrophage has its origin in the circulating blood monocyte. Its importance in pleural space maybe as a modulator of pleural injury, being called to the pleural space by chemotactic agents released by PMNs. It appears to be important in localizing pleural injury whether due to micro -organisms or chemicals. An abundance of macrophages leads to localized pleural space fibrosis. The presence of pleural macrophages is of no diagnostic value but differentiation from mesothelial cells is important since the presence of macrophages does not exclude tuberculous pleurisy.

From the practical point of view cellular effusions characterised by severe lymphocytosis and the absence of mesothelial cells should be considered as tuberculous or less frequently neoplastic [9], until proved otherwise.

We have also found the increase in the number of lymphocytes in our tuberculous exudates (83 ± 1.6). The controls from other conditions show comparatively less number of lymphocytes (17±13.16). Two malignant samples show more than 50 % lymphocytes whereas the other 3 samples of bronchial pneumonia show more of PMN (81±0.71).

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Pleural biopsy has been introduced recently as a diagnostic procedure and has been found to be promising. However even in proved cases of tuberculosis the first pleural biopsy is diagnostic only in about 60% of the cases.

Conclusion –

The importance of the protein content of the pleural effusion has been stressed for a long time. It can be concluded from the result obtained that lymphocyte count along with the protein estimation has more diagnostic value as far as tuberculosis exudate is concerned.

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