Dissertation on

Comparison of Pleural Fluid Cholinesterase estimation Vs Abbreviated Lights Criteria in Diagnosing Pleural Effusions

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Certificate

This is to certify that the dissertation "COMPARISON OF PLEURAL FLUID CHOLINESTERASE ESTIMATION VS ABBREVIATED LIGHTS CRITERIA IN DIAGNOSING PLEURAL EFFUSIONS" is the bonafide original work of Dr.R.Logamurthy in Partial fulfillment for M.D.BRANCH-XVII (T.B. AND RESPIRATORY DISEASES) EXAMINATION of The Tamilnadu Dr. M.G.R.University to be held in September 2006. The period of study was from 2003 to 2006.

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DECLARATION

I, **Dr.R.Logamurthy**, declare that dissertation titled **Comparison of Pleural fluid estimation Vs Abbreviated Lights criteria in diagnosing pleural effusions**" is a bonafide work done by me at Institute of Thoracic Medicine, Chetput and Department Of Thoracic Medicine, Madras Medical College & Govt. General Hospital, Chennai-3 under the guidance of my Professor **Dr.R. Atharunnisa Begum M.D. (T.B. &C.D)**

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CONTENTS

BACKGROUND

Pleural effusions are collections of fluid in the pleural spaces. The major function of the pleura and the pleural space is to permit the lungs to expand and deflate within the chest. Since the pleural space is subatmospheric with no barrier to liquid movement the space can occupy large volume of fluid. These fluid collections (Pleural effusions) are very common clinical entity.

These abnormal collections of pleural fluid in the pleural spaces are either because of an increased entry of pleural fluid into the space, a decrease in exit of fluid from the space or both. These effusions are quite common clinical problems and they are associated with a myriad of clinical diseases.

Traditionally pleural effusions are classified into exudates and transudates after thoracentesis. Two thirds of pleural effusions are caused by congestive cardiac failure, pneumonia, malignancy and pulmonary embolism according to literature available. It is of considerable clinical importance to differentiate between exudates and a transudates as the latter does not need any treatment . Transudates resolves by itself where as an exudative pleural effusion clearly needs an intervention.

Pleural exudates are secondary to alterations in the capillary permeability of the pleura or lymphatic clearance where as transudates are due to alterations in the hydrostatic pressure , alterations in the colloid

osmotic pressure in the capillaries and fluid entering the pleural cavity from the peritoneal cavity through defects in the diaphragm or lymphatics. Therefore determining whether the fluid is exudative or transudative is the first step in diagnosing pleural effusions of unknown etiology. In order to differentiate between the two, many biochemical parameters have been used like protein, sugar, LDH, Bilurubin, cholesterol, ADA, and their respective ratios with their serum values have been used. A protein level of 3gms/dl and LDH level of 200 IU has used as the dividing line between exudates and transudates.

Many criteria's have been used to discriminate between exudates and transudate. The one which has used more frequently is the criteria introduced by Lights *et al*[1] in 1972. It was the one which showed improved diagnostic efficacy over the other criteria.

Lights criteria[1] includes 3 parameters, they are

1) Pleural fluid to serum protein ratio > 0.5

2) Pleural fluid to serum LDH ratio > 0.6

3) Pleural fluid LDH >200 IU of upper limits of normal.

Then later it was found that lights criteria misclassified about 20 to 30% of pleural effusions [3]. To reduce the misclassifications some modifications were made in the lights criteria. Other investigators have examined the diagnostic utility of tests that measure pleural fluid cholesterol

bilirubin and albumin. But they also misclassified significant number of pleural effusions and their superiority over the lights criteria in discriminating pleural exudates and transudates remained doubtful.

Cholinesterase has been used by a few workers for differentiation between pleural exudates and transudates.Cholinesterase activity is significantly inhibited in presence of untreated Pulmonary Tuberculosis [3] and its activity remained stable in any one individual. Therefore measurement of its activity may serve as useful diagnostic tool.

In 1978 Cabrer *et al*[16] estimated Cholinesterase activity in pleural effusions of various etiologies and concluded that there exists differences in activity of Cholinesterase and it was possible to differentiate transudate and exudates.

In 1996 Eduardo *et al*[2] concluded that Pleural fluid to serum Cholinesterase ratio was the most accurate criteria for separating exudates and transudates and suggested that it should be used as the first step is diagnosis of pleural effusions, if further studies confirmed their results.

In 1996 Garcia and Padilla[4] confirmed the importance of estimation of Cholinesterase activity in the diagnosis. In 1999 A.K.Janmeja, .M.Goyal and Manju Sharma[3] concluded that Cholinesterase ratio has a better discriminative capacity than lights criteria. The literature available on this study is less and therefore this study was planned to find out the efficacy of cholinesterase ratio in classifying pleural effusions and then comparing it with Abbreviated Lights Criteria (Pleural fluid to serum protein ratio).

AIMS OF THE STUDY

1) To evaluate the efficacy of Pleural fluid to Serum Cholinesterase Ratio in diagnosing pleural effusions.

2) To Compare Pleural fluid to Serum Cholinesterase Ratio with Abbreviated Light's Criteria.

A REVIEW OF LITERATURE

Pleural Effusions

Pleural Effusions has been known to clinical medicine since ancient times. Ancient texts of Indian Medicine-Ayurvedas had mentioned about drugs named “Kalyana sundara Rasha” which was found to be effective in treating conditions that caused water accumulations in the chest. But there was no direct mention of pleural effusions as such.

Ancient Egyptian textbooks of medicine found in pyramids “Ebers Papyrus” now in the Library of Leipzig had a mention of adhesions of pleura in one case. Hippocrates (460-361BC) knew this entity which he thought as an inflammation of the lungs including the pleurae and termed it as parapneumonia. This was then known for many centuries to come. He also described Tuberculosis as Phthisis.

Castiglioni, while reviewing other ancient texts found mention of clinical findings like noisy chest as if rubbing of leather straps, succession splash and snoring sounds in cases of pus in the chest while shaking the chest. These were diagnosed by applying ear to the chest directly.

Avicenna’s “Canon of Medicine” which was translated into Latin and had been a foundation course of medicine in Universities of Europe from 1250 AD to 1600 AD had mentioned about pleurisy which says that the signs of simple pleurisy are clear, fever is continuous, and there is sharp pain beneath the ribs, which sometimes is only felt when the patient breathe

strongly. The third sign is difficulty and frequency of respirations, the fourth is a rapid and weak pulse rate, the fifth sign is the cough which at first is dry and then is accompanied by sputum.

Thomas Sydenham considered parapneumonia similar to pleurisy except that it affected the lungs more. He was later called "Peripneumonia Notha" by his pupils. The adhesions of the surfaces of both pleurae (Brock Bank) was described by Geovani Battista Mergagni . John Huxham in his essays of fever: because of it's sluggish course and small fever and heat in contradistinction to pleurae- Para pneumonia where severe chest pain, acute high fever, breathing difficulty and spitting of blood was marked. In 1778 Herman Boerhave noted in his "Aphorism", that pleurisy is different and associated with pain which is increased on deep breathing and site of pain is the pleura. William Cullen again described all inflammation under the heading pneumonia .He emphasized that though sometimes distinct yet in general no accurate limits can be placed between pneumonia and pleurisy.

Leopold Joseph Avenbrugger(1722-1809) invented chest percussion as a diagnostic procedure . His published work, entitled *Invention Novum* became a landmark in history of medicine. But his works importance remained unknown until Jean Nicholas Covisart (1755-1821) published a translation and commentary in 1808.

Rene Theophile Hyacinthe Laennec(1781-1826) through his invention of the stethoscope in 1816 gave us the modern concept of pneumonia , pleurisy , pulmonary tuberculosis and other pulmonary condition . He published his book on auscultation on 1819, titled *Del' Auscultation Mediate*

etc. This book introduced a diagnostic tool into medicine for the first time. He also wrote one of the most clear, accurate and almost completes account of chest diseases.

Joseph Skoda (1805-1881) in 1839 added another useful diagnostic point to auscultation and percussion Skodiac resonance was described in relation to lobar pneumonia and over the air containing upper lobe above the level of a consolidated zone or the level of effusion (Brock Bank). Bowditch (1808-1892) was the first to tap pleural effusion.

Pleura – Anatomy & Pathophysiology

The Pleura consists of a single layer of mesothelial cells (mesothelium) supported by the submesothelial connective tissue. . There are two pleural sacs, one on the either side of the mediastinum. Each sac is invaginated from its medial side by the lung, so that it has an outer layer the parietal pleura and an inner layer, the visceral or pulmonary pleura. The layers are continuous with each other around the hilum of the lung and enclose between them the potential space of pleural cavity with a negative pressure. The normal pressure is -2 to -6mm 0f Hg.

The pleural space serves as a coupling system between the lung and the chest wall. The thin rim of the fluid that normally separates the parietal from the visceral pleura is thought to facilitate the movements of the lung within thoracic cavity.

The visceral pleura are more vascular than the parietal pleura. The later has sensory nerve receptors. Both the pleura can be the site of infection but usually pleural cavity and its surfaces secondarily involved by the diseases of the adjacent structures which arise from the heart, chest wall and the mediastinum, it is also involved in subdiaphragmatic pathology(subphrenic space, pathology of liver, spleen, pancreas , kidney etc.,

The pleura is not just a simple envelope protecting the lung and acting as a permeability barrier, cells in the pleural space cooperate to maintain homeostasis through an intricate network of interactions. Particles, microorganisms and metastatic cells are able to alter the homeostatic state, resulting in the development of pleural effusions, fibrosis and neoplasms.

The normal pleural fluid production is about 0.16-0.36ml/kg of body weight in each hemithorax. The systemic blood supply of the parietal pleura is thought to be the major source of normal pleural fluid. The parietal pleural microvessels are closer to the pleural space than those of the visceral pleura. The parietal pleural microvessels have a higher microvascular pressure because of their drainage into the systemic venules, while the visceral bronchial vessels drain into the low resistance pulmonary vessels. The liquid and the protein filtered from the pleural microvessels can flow across the mesothelial layer along the pressure gradient into the pleural space.

The pleural space, by virtue of its size and surface area, its subatmospheric pressure, and its relative leaky borders, is clearly vulnerable to the accumulations of liquids.

Studies show that pleural fluid absorption occurs by bulk flow and not by diffusion. Compared with the visceral pleura the parietal pleura is more permeable to liquids and particles. The only possible exit of the pleural fluid is via parietal pleural stomata which directly link pleural fluid to pleural lymphatics.

Pleural fluid analysis

Pleural effusions can be caused by diseases in the chest, organs dysfunction or infections below the diaphragm, drugs and systemic diseases. In the U.S. approximately 2/3rd of the pleural effusions are due to CCF, pneumonia, malignancy and pulmonary embolism. In our settings most of the cases are due to infectious causes particularly due to tuberculosis. This may also be due to underdiagnosis of above mentioned conditions.

Clinicians may first suspect a pleural effusion with the history, physical examination or by a chest x-ray film PA view. Then a lateral decubitus film or an ultrasound chest is done to confirm the findings. The next step is to do a diagnostic thoracentesis blind or guided. Here again not all cases need diagnostic thoracentesis. If the patient has classical presentation of CCF or CRF the effusion resolves with treatment of primary causes.

After thoracentesis the first step is to observe the colour, character and odour of the fluid. These will be helpful in making an initial diagnosis. A clear straw colored fluid suggests a transudate, but it can also be seen in

paucicellular exudates. A serosanguinous appearance signifies a pleural fluid hematocrit of <1%. A gross bloody effusion narrows the differential diagnosis to malignancy, Benign Asbestos pleural effusion, Post Cardiac Injury Syndrome, pulmonary infarction, and trauma.

A haemothorax occurs most commonly in trauma, but can be also seen with invasive procedures, metastatic disease to pleura, and anticoagulation in pulmonary infarction and catamenial haemothorax. To diagnose haemothorax, pleural fluid haematocrit is compared with blood haematocrit which should be atleast 50% of the peripheral blood haematocrit. Then ICD is usually necessary.

Withdrawal of milky or white fluid from the pleural space points towards either a chylothorax or a cholesterol effusion. Empyema can also simulate these conditions. To differentiate the empyema and lipid effusion centrifugation of the fluid is helpful. The former the cells settle down with a clear supernatant fluid in the top, while the latter will remains white.

A longstanding bloody effusion may appear brown while brownish viscous fluid may represent an amoebic liver abscess rupturing into pleural space. A black pleural fluid may be due to Aspergillus Niger infection, whereas a yellow-green tinted fluid may be due to rheumatoid pleural effusion .

Anchovy paste appearing pleural fluid is virtually diagnostic of hepatopulmonary amoebiasis. When the pleural fluid smells like ammonia

the diagnosis of urinothorax is established which is caused by obstructive uropathy.

Determining whether the fluid is exudate or transudate is the first step in pleural fluid analysis, when evaluating patients with pleural effusion of unknown etiology.

Exudative pleural effusion is caused by broad array of conditions which includes infections, neoplasms and inflammatory conditions. In contrast, transudative pleural effusion is caused by limited number of conditions; majority of which results from hydrostatic mechanism.

Causes of Exudative Pleural effusion

Infectious causes:

Bacterial pneumonia
Myco.tuberculosis
Fungal pneumonia
Atypical pneumonia
Actinomycosis
Nocardiosis
Hepatic abscess and hepatitis
Pancreatitis

Malignant conditions:

Carcinoma
Lymphoma

Leukemia

Mesothelioma

Inflammatory conditions:

BAPE (Benign Asbestos pleural effusion)

Pulmonary infarction

Sarcoidosis

PCIS (Post Cardiac Injury Syndrome)

ARDS

Uraemic pleurisy

Pancreatitis

Haemothorax

Increased Negative pleural pressure:

Atelectasis

Trapped lung

Cholesterol effusion

Connective tissue disorders:

Lupus pleuritis

Rheumatoid pleurisy

MCTD

Churg Strauss syndrome

Wegeners Granulomatosis

Endocrine dysfunctions:

Hypothyrodism

Ovarian hyperstimulation syndrome

Lymphatic abnormalities:

Malignancy

Yellow Nail syndrome

LAM

Lymphangiectasis

Movement of fluid from abdomen to pleural space:

Acute Pancreatitis

Pancreatic Pseudocyst

Meigs syndrome

Carcinoma, Chylous Ascites,

Urinothorax

Causes of Transudative Pleural effusion:

Congestive Cardiac Failure

Cirrhosis

Nephritic syndrome

Peritoneal Dialysis

Hypoalbuminaemia

Atelectasis

Constrictive Pericarditis

Trapped Lung

SVC Obstruction

It is important to discriminate Exudates and transudates accurately. Because of the prognostic significance of the conditions causing exudative

effusion, the detection of exudates warrants additional diagnostic studies for most patients to determine the underlying cause of fluid formation.

Conversely, a patient's general clinical presentation is usually sufficient to determine the cause of transudative effusion without further diagnostic studies.

Exudative Pleural effusions are defined by the presence of a high concentration of large molecular weight constituents when compared to transudative effusions. Tests that determine the presence of exudates measure one or more of the constituents directly or by their effects on pleural fluid specific gravity. Tests results have been used singly or in combination. The use of test in combination derives clinical importance of maximizing the sensitivity of the testing strategies to enhance the detection of exudates. Exudative effusions often signify the presence of clinically occult conditions that may have considerable prognostic significance. Therefore a screening strategy is employed wherein misclassification errors favours the overdiagnosis of exudative pleural effusions. As a rule, combination tests have a higher sensitivity, but a lower specificity than a single test strategy, even if single tests have similar diagnostic properties to the individual test components within the combination strategy.

Initially pleural fluid specific gravity and pleural fluid protein measurement was used to detect exudative pleural effusions, later to increase the sensitivity Lights & coworkers established what is now known as "Lights criteria"[1] The three original criteria proposed were

4) Pleural fluid to serum protein ratio > 0.5
5) Pleural fluid to serum LDH ratio > 0.6
6) Pleural fluid LDH >200 IU of upper limits of normal.

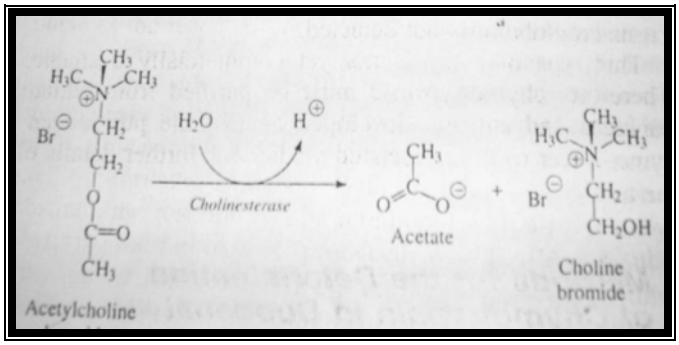
Since different laboratories report different normal ranges of LDH, the last criteria was later modified to an LDH concentration of greater than 2/3^{rds} of the upper limits of normal for the laboratory's serum LDH test result. Later it was found that lights criteria had a sensitivity of 98% and specificity of 74% in identifying exudative pleural effusions from validation studies using pooled data from several primary investigations. In the recent years, other test having proposed to indicate a pleural exudates have included a pleural fluid cholesterol > 60 mg / dL [10,11] . A pleural fluid cholesterol > 45 mg / dL [12], a gradient of less than 1.2 g / dL for the difference in the pleural fluid and serum albumin level [13] , a pleural fluid to serum bilirubin ratio above 0.6 [14] , and a pleural fluid to serum cholinesterase ratio above 0.23 [4,7] .

Cholinesterases: a review

Two related enzymes have the ability to hydrolyze acetylcholine. One is acetylcholinesterase, which is called true cholinesterase or choline esterase1.true cholinesterase is found in erythrocyte, lung, spleen, nerve endings and the grey matter of brain. It is responsible for prompt hydrolysis of acetylcholine to release at the nerve endings to mediate transmission of neural impulse across the synapse. The degradation of acetylcholine is necessary to the depolarization of the nerve so that it can be repolarised in the next conduction event.The other cholinesterase is acylcholine acylhydrolase; it is usually called pseudocholinesterase, benzoyl

cholinesterase or cholinesterase 2.Although it is found in the liver, pancreas, heart, white mater of the brain and serum, its biological role is unknown. The serum enzyme is the one whose assay is clinically useful.

The type of reaction catalyzed by both cholinesterases is



The two enzymes differ in specificity toward some substrate while behaving similarly toward others. The serum enzyme acts on benozylcholine but cannot hydrolyze acetylB- methylcholine; the red cell enzyme acts on the latter but not on the former. Only choline esters are split by the red cell enzyme; aryl or alkyl esters are not attacked. The red cell enzyme is inhibited by its substrate, acetylcholine, if present at concentrations about the 10-2 mol/liter; the serum enzyme is not inhibited by the substrate.

Both enzymes are inhibited by physostigmine and prostigmine, both of which contain quaternary nitrogen in their structures. These two compounds are typical competitive inhibitors, competing with the choline residue of acetylcholine for its binding site on the enzyme surface. Both the enzymes are irreversibly inhibited by the some organic phosphorus compounds, such as diiso-propylflurophosphate.The phosphoryl group binds

very tightly to the enzyme site at which binding of acyl group normally occurs, thus preventing the attachment of acetylcholine. Both enzymes are inhibited by large variety of other compounds, among which are Morphine, Quinine, tertiary amines, citrate, borate and bile salts. The cholinesterase present in the normal sera can be separated by electrophoresis into 7 to 12 bands, the number obtained depending upon the experimental technique used. The isoenzymes of acetylcholinesterase differ in molecular size and appear to be aggregates of different numbers of the same basic unit. Of more interest are the typical variants of the enzyme, characterised by the diminished against acetylcholine and the other substrates, which are found in the sera of a small fraction apparently healthy people. The gene controlling the synthesis of SChE can exist in many allele forms. Four of the most common forms are designated as Eu1,Ea1,Ef1and Es1.Atleast 25 other forms exist, and another gene locus is recognized (E2).The normal, most common phenotype is designated as Eu1Eu1,or UU.The gene Ea1 is referred to as the atypical gene; the sera of people homozygous for this gene (Ea1Ea1,=AA) are only weakly active toward most substrates for cholinesterase and poses increased resistance to inhibition of enzyme activity by dibucaine. The Ef1 gene also gives rise to a weakly active enzyme but with increased resistance to fluoride inhibition. The Es1 gene (s for silent) is associated with absence of enzyme or the presence of a protein with minimal or no catalytic activity. The mutations that give rise to the typical and fluoride-resistant Cholinesterase variants involve a change in the structure of the active center. The variant isoenzymes (allelozymes) are less effective catalysts the usual form; the affinity of the enzymes for the substrates is reduced (i.e., Km is increased), and affinity for competitive inhibitors, such as dibucaine or fluoride, is similarly decreased. This gives

rise to the characteristic dibucaine or fluoride resistant properties of the genetic variants that are exploited in their characterization. The homozygous forms, AA or FF, are found in only 0.3 to 0.5% of the white population; heir incidence among blacks is even lower. Inheritance of increased. Cholinesterase activity has also been reported in a few families. This is apparently due to increased production of the usual allelozyme.

CLINICAL SIGNIFICANCE:

Cholinesterase levels in serum are useful as a test of liver function, as an indicator of possible insecticide poisoning, or for the detection of patients with atypical forms of the enzyme. The spread of values encountered in apparently healthy people is rather wide, ranging between 4000 and 12000 U/L at 37.C.The level in any given person is fairly constant; a fall in enzyme level of greater than 670U/L is significant at the 95% confidence level. Levels at birth are only one-fourth those of adults but reach adult levels by the second month of life. No enzyme is found in urine. Measurements of Cholinesterase activity can serve as sensitive measures of the synthetic capacity of the liver if a patient's normal (baseline) level is known, which unfortunately the case is rarely. In the absence of known inhibitors, any decrease in activity in serum reflects impaired synthesis of the enzyme by the liver. A 30 to 50% decrease in level is obtained in acute hepatitis and in chronic hepatitis of long duration. Decreases of 50 to 70% occur in advanced cirrhosis and carcinoma with metastases to the liver. Essentially normal levels are noted in chronic hepatitis, mild cirrhosis, and obstructive jaundice.

Decreased levels of serum enzymes are also found in patients with acute infections, pulmonary embolism, and muscular dystrophy, as well as after surgical procedures. After a myocardial infarction, the enzyme level decreases until the fifth day and then begins a slow rise to normal. Decreased levels are also found in chronic renal disease and in pregnancy.

A marginal increase in enzyme levels may be observed in patients with nephrotic syndrome. Synthesis of albumin to replace that lost in the urine is accompanied by some synthesis of additional cholinesterase, although syntheses of the enzyme and albumin by the liver are independent of each other. Marginal increases in enzyme are also seen in thyrotoxicosis and hemochromatosis, in obese diabetic people, and in patients with anxiety and other psychiatric states.

Among the organic phosphorus compounds that inhibit cholinesterase activity are many organic insecticides, such as Parathion, Sarin, and tetraethyl pyrophosphate. Workers engaged in agriculture and those working in organic chemical industries may be subjected to poisoning by inhalation of these materials or by contact with them.

Obviously, if enough material is absorbed to inactivate all the acetylcholinesterase of nervous tissue, death will result. Both cholinesterases are inhibited, but the activity of the serum enzyme falls more rapidly than does that of the red blood cell enzyme. A 40% reduction in serum enzyme activity occurs before the first symptoms are felt, and a drop of 80% is required before serious neuromuscular effects become apparent. Near-zero

or zero levels pf enzyme activity require emergency treatment with such enzyme reaactivators as pyridine-2-aldoxime.

Succinyldicholine (suxamethonium) and mivacurium are drugs used in surgery as muscle relaxants. Because succinyldicholine is very similar to acetylcholine, it is also hydrolyzes by cholinesterase, and its physiological effect persist only long enough (30-50min) to meet the needs of the surgical procedure. In patients with low levels of enzyme activity or in those with the atypical, weakly active enzyme variants, destruction of this drug does not occur rapidly enough, and patients may enter a period of prolonged apnea requiring mechanical ventilation until the drug is eliminated by other routes. Preoperative screening has been advocated to identify patients in whom suxamethonium administration may lead to complications. However, more often, the laboratory is asked to investigate patients and their relatives in whom suxamethonium sensitivity has been found.

The degree of sensitivity varies with the phenotype of the patient and the dose of the drug, size of the patient, and duration of surgery. There is a decline in total activity from individuals who are homozygous for the usual allele, through those who are heterozygous for the usual and a variant allele, those who are homozygous or heterozygous for variant allele, to zero in subjects in whom two “silent” alleles are paired. Subjects who poses one normal allele (i.e., who are heterozygous for the normal and variant allele) usually produce enough enzyme to protect them against suxamethonium sensitivity, whereas patient with paired variant allele (either as homozygote or heterozygote) shows various degree of sensitivity.

Measurements of total SChE activity and determination of the "dibucaine number" and "fluoride number" are needed to characterize cholinesterase variants fully. The later parameters indicate the percentage inhibition of enzyme activity towards specified substrates in the presence of standard concentrations of these reagents. The average values of the dibucaine number for normals, heterozygotes and homozygotes (Ea1 gene) are 78, 60 and 16% respectively, when benzoylcholine is used as substrate. Dietz and associates reported values of 81 to 86, 67 to 80, and 8 to 35% respectively, with propionylthiocholine (PTC) as substrate. Analogous fluoride numbers with PTC as substrate are 77 to 81, 67 to 79 and 54 to 65% respectively Phenotypes most susceptible to apnea after suucylcholine administration are AA,AS,FF,FS,SS,AF and to some extent UA.

Cholinesterase levels in cerebrospinal fluid are very low. The total activity present in fluids from healthy individuals is about 17+/-4 U/L.Both the serum and red blood enzyme forms can be detect ed, with the serum form present in the greater concentration. Elevation of Cholinesterase activity are appreciable in diseases involving destruction of brain parenchyma, in brain tumors and in brain abscess, in hydrocephalus (to 40U/L), in Guillain-Barre disease (to 60U/L), and often in meningitis and multiple sclerosis. In other neurological and non neurological diseases, enzyme levels are normal or any changes are too nonspecific and inconsistent to be of mush value in diagnostic work. Cholinesterase activity is independent of routine concentration and of leukocyte count, the leukocyte containing no enzyme activity.

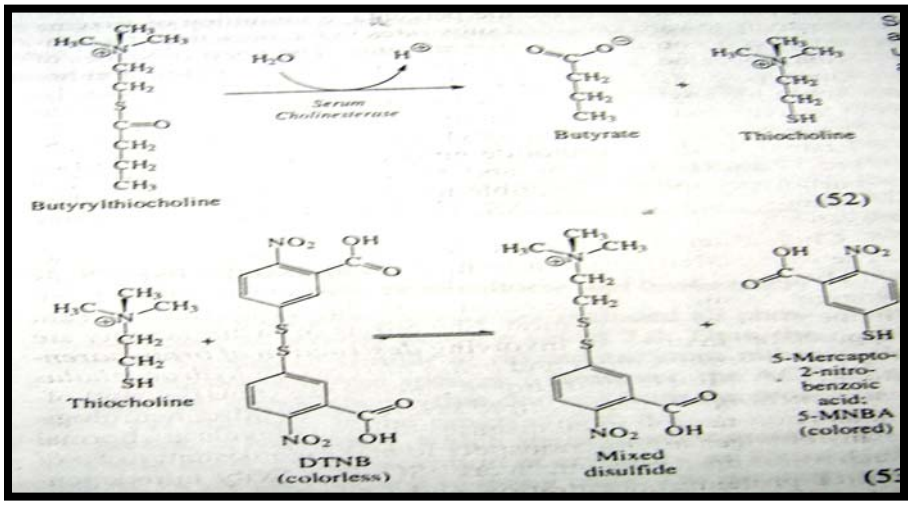
METHODS FOR DETERMINATION OF CHOLINESTERASE ACTIVITY

The hydrolysis of acetylcholine, the substrate used in older methods, results in the formation of 1 mol of hydrogen ions for each mol of substrate reacted. In manometric method, these hydrogen ions react with bicarbonate buffer to release carbon dioxide, which is then measured. In the potentiometric procedure of Michel, the decrease in pH during the course of a fixed reaction period is measured. The acid formed can be titrated with a pH-stat. If pH indicators that change colour in the pH range of 6-8.5 (bromothymol blue, m-nitrophenol) are incorporated into the reaction mixture, the change in colour, measured spectrophotometrically, can serve as a measure of enzyme activity. A number of kits and test papers devised for testing for enzyme activity in the field are based on this principle.

In the procedure of de la Huerga and colleagues, the quantity of untreated acetylcholine remaining after a 60 min reaction period at 37.C is measured. The ester is reacted with hydroxylamine to form the hydroxamate derivative; the complex forms an orange-brown complex with ferric ions in acid solution that can be measured photometrically at 540nm. Kalow and Genest proposed benzyl choline as substrate and measured the decrease in sorbents of the substrate at 240 nm.

Contemporary methods used acylthiocholine esters as substrate. The substrate are hydrolyzed at approximately the same rate as cholineesters,and the thiocholine formed can be measured by reaction with chromogenic disulfide agents such as DTNB (Ellman's reagent)[22] or 4,4'-dithiodipyridine.

The iodide salts of acetyl-, propionyl-, and butryl thiocholine all have been used as substrate. The reactions for butryl thiocholine with DTNB as the chromogen are given in equations.



The DTNB reaction produces a color that can be measured at 410nm. If 4,4'-dithiodipyridine is used as a chromogen, mercapopyridine is formed; this immediately tautomerizes to thiopyridine, which absorbs at 344nm.

DETERMINATION OF SERUM CHOLINESTERASE ACTIVITY

Principle:

Activity of SChE is determined from the rate of hydrolysis of propionyl-thiocholine in the presence of DTNB. The reaction of thiocholine product with colourless DTNB forms coloured 5-MNBA, which is measured spectrophotometrically at 410 nm. Dibucaine or fluoride inhibition can be estimated by performing concurrent assays in which dibucaine or fluoride is present in the substrate mixture. Percent inhibition is evaluated by

comparison of activity in the inhibited system with that of uninhibited system.

Specimen:

Serum is the sample of choice. Enzyme activity is stable for several for several weeks whether the specimen is stored at room temperature or under refrigeration. Moderate hemolysis does not interfere if separated serum has been centrifuged to remove RBC ghosts.

Reagents:

1. Phosphate buffer, pH 7.6, ionic strength 0.1 in water.
2. Propionylthiocholine iodide (PTCI), 20mmol/L in water.
3. Dibucaine, 0.3mmol/L in water.
4. Sodium fluoride, 40mmol/L in water.
5. DTNB-buffer (Color Reagent). 0.423mmol/L in Phosphate buffer.
6. Substrates:
    a. Uninhibited reaction. Mix equal parts of PTCI (Reagent 2).
    b. Dibucaine-inhibited reaction. Mix equal parts of PTCI and Dibucaine Solution (Reagent 3).
    c. Fluoride- inhibited reaction. Mix equal parts of PTCI (Reagent 2) and Fluoride Solution (Reagent 4).
7. Quinidine sulfate, 0.5 g/dl in water.

Final concentrations in assay mixtures, in mmol/L, are PTCI, DTNB, 0.254; phosphate, 25; and (when present) dibucaine, 0.03 or fluoride, 4.

Procedure:

1. Dilute serum 100-fold with water.
2. For each inhibited or uninhibited assay, prepare two 16x 125-mm tubes, labeling one B (blank) and U (unknown).
3. Add to U and B tubes, 3.0mL DTNB-buffer and 1.0 mL of appropriate substrate mix.
4. Warm tubes at 37deg C for 5 min.

5. Without removing the tube from the bath and starting timer on addition, add to u tube 1.0 mL of diluted serum. Mix and allow remaining at 37deg C for 3 min.
6. Add, to U and B tubes, 1.0 mL Quinidine sulfate. Mix.
7. Add to tube B, 1.0 mL diluted serum and mix.
8. As color quickly fades, transfer contents of corresponding U and B tubes to 1 –cm cuvets and proceed promptly to read absorbance.
9. Read absorbance of U against B set to zero absorbance at 410 nm. Under these circumstances absorbance of U represents absorbance ΔA/3 min.

Calculations:

Uninhibited reaction:

$$U/L = \frac{\Delta A/min}{13.6 \times 6.0/0.01} = \Delta A/min \times 14710$$

Where

$\Delta A/min = Au/3\ min$

13.6 = L x μmol -1 x cm-1, the micromolar absorption coefficient of 5-MBNA.

6.0 = total value of reaction system (mL).

0.01 = volume of serum in reaction system

Percentage of inhibition

% of inhibition = (1- Au of inhibited system / Au of uninhibited system) x 100.

Comments:

The original report describes alternatives of timing, temp and standardization.

Reference intervals for genetic variants are also presented.

Reference Interval:

For the genotype Eu1Eu1, the mean +/– SD for foregoing method is 8440 +/ – 1780 U/L.

Reference:

Dietz A.A., Rubinstein, H.M., Lubrano, T.: colorimetric determination of serum cholinesterase and its genetic variants by propylthiocholineedthiobis (nitrobenzioc acid) procedure. .Clin. Chem 19:1309-1313, 1973.

MATERIALS AND METHODS

This study is a Prospective case study undertaken during a period of 8 months (Feb 2005-Sep 2005) done at Institute of Thoracic Medicine, Chetput, chennai-31 & Dept of Thoracic Medicine, Madras Medical College and Govt.General Hospital, Chennai-3 .

The criteria for the patients taken up for study are as follows:

Inclusion criteria:

1. Tuberculosis pleural effusions
2. Malignant Pleural effusions
3. Para pneumonic effusions
4. Effusions with cardiac failure
5. Effusions with renal failure

Exclusion criteria:

1. Pleural effusion with more than one etiology
2. Pleural effusion in patients with hepatic disease
3. Pts on Oral contraceptives
4. Pts on anticancer drug, MAOIs, Neostigmine
5. Pts on chlorpromazine

6. Pleural effusion in patients pregnant
7. Pts with H/O exposure to pesticides.
   (organophosphorous compounds)

A study population 80 patients with clinical and radiological evidence of Pleural effusion of diverse etiology were studied after exclusion. A relevant history and detailed clinical examination was done. The following investigations were done.

Blood: Hemoglobin, Total count, Differential count, ESR.
Routine urine examination.
Chest Roentgenogram PA and Lateral View.
Sputum for AFB smear.
Sputum Cytology for Malignant Cells.
Mantoux test.

Then **Thoracentesis** was performed in each case with due aseptic precautions. Care was taken not to let fluid to mix with blood .About 10mL to 15mL of pleural fluid was aspirated in each case and Specimen was stored at 2^0-8^0 Celsius. Within half an hour 10ml blood was collected in vaccutainer and centrifuged and sera stored at 2^0-8^0 Celsius.

The pleural fluid was analyzed as follows

1. Initially color of the fluid was noted.
2. Pleural fluid was sent for biochemical analysis for protein, sugar, LDH.

3. Pleural fluid was sent for microbiological analysis for Grams stain, AFB smear and Culture and NT culture in selected cases.
4. Pleural fluid was sent for cytology for Malignant Cells.
5. Then cholinesterase activity in the pleural fluid and sera was assayed by the methods described by Dietz [6] by using Randox Butyrylcholinesterase kit by kinetic calorimetric method (Ellman's reaction [22] .).
6. Similarly the serum protein, sugar, LDH and cholinesterase activity was analyzed.

OBSERVATION AND RESULTS

In the present study 80 cases of patients who had pleural effusions of diverse etiology was selected. The clinical, radiological and relevant laboratory investigations and diagnostic pleural aspiration were done in all these cases. The pleural fluid was analyzed by looking for the color of the fluid, microscopy, cytology and relevant biochemical parameters which included the estimation of serum cholinesterase and pleural fluid cholinesterase.

The patients were divided into 5 groups depending on the etiology of the pleural effusion. They are tuberculosis, malignant, parapneumonic, CCF, and CRF, the first three being exudates and the last two transudates. The criteria for tuberculous effusion included a clinical presentation, history of contact, radiological evidence, and sputum for AFB and culture. For transudative effusions cardiac and renal decompensation was taken into account. The inclusion criteria were pleural effusions with protein content less than 3 Gm% and no evidence suggestive of inflammatory or infectious cause.

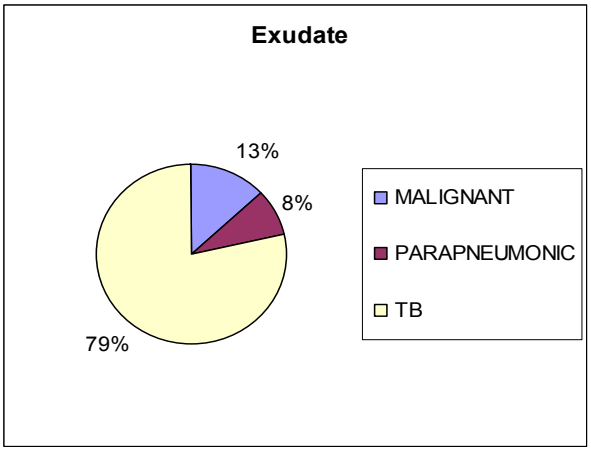
For the malignant group, the clinical presentation, age, sex, smoking habit, loss of weight, clubbing, color of the pleural fluid, rate of reaccumulation, and cytopatholgical and histopathological confirmation was looked for. For the diagnosis of effusion in pneumonia cases age, sex, onset of the fever, color of the pleural fluid, cell count, presence of pus cells and organisms were taken into account.

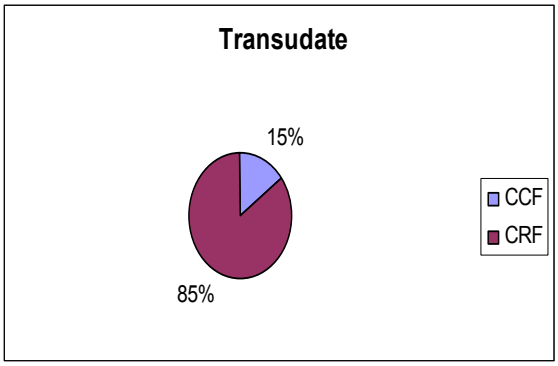
Etiological Group and their Distribution

Table 1

Group	Type	No of cases
A	Tb	47
B	Malignant	8
C	Parapneumonic	5
D	CCF	3
E	CRF	17

- Exudates 60 (A,B,C)
- Transudates 20 (D,E)





Out of the 47 cases in group A, 33 cases complained of breathlessness, 28 cases complained of fever, 6 cases had haemoptysis, 32 had cough. In group B, 4 cases complained of breathlessness, one case had fever, all the cases had cough and 2 cases had story of hemoptysis. In group C all the cases had History of fever, 4 cases had cough, and 2 cases had breathlessness. In groups D and E breathlessness and cough were the predominant complaints. The signs were as shown in the table below

Table 2

SIGNS	A	B	C	D	E
CLUBBING	-	3	-	-	-
ANEMIA	20	4	1	2	17
PEDAL EDEMA	-	1	-	3	15
LYMPHADENOPATHY	-	2	-	-	-

On analysis of the cell count it was found that WBC count was within 4000 to 10000 cells/cu mm in group A and in Group C out of 5 cases 4 cases had WBC count more than 10000 cells/cu mm.

Table 3

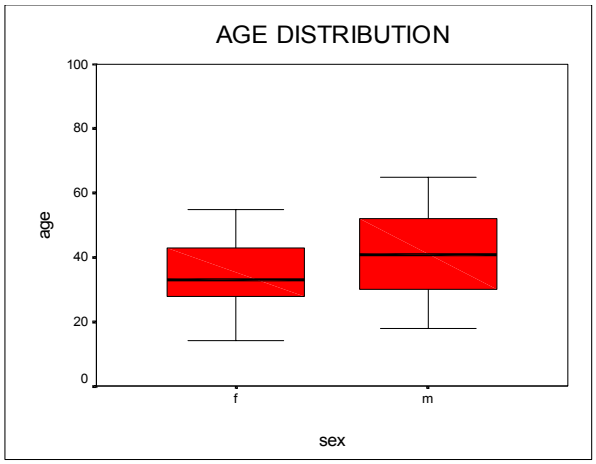
Total WBC Count	A	B	C	D	E
> than 4000 cells	-	-	-	-	-
4000-10000 cells	47	8	1	-	-
>than 10000 cells	-	-	4	-	-

Then ESR was found to be elevated in Group A, B, and C groups, the highest being in the group B. It was measured using Westergren's measured. Mantoux test was positive in all the cases in group A.

Sputum examination was done in each case for AFB and malignant cells. For AFB smear sputum was collected according to RNTCP guidelines i.e. Spot-early morning- spot. It was positive in 3% of suspected tuberculous pleural effusion cases. For the detection of malignant cells in sputum was 3 sputum specimens collected in 70% alcohol on successive 3 days and sent for cytological analysis by pap method. In this study about 2 cases turned out to be adenocarcinoma.

Chest Roentgenogram was done in all cases for the confirmation of clinical diagnosis. Pleural fluid examination was done in all the cases. First of all the color of the fluid was noted if there was any bleeding noted then the first sample was discarded and then the next sample was taken for analysis.

Age distribution shown in the figure below:



Color of Pleural Fluid:

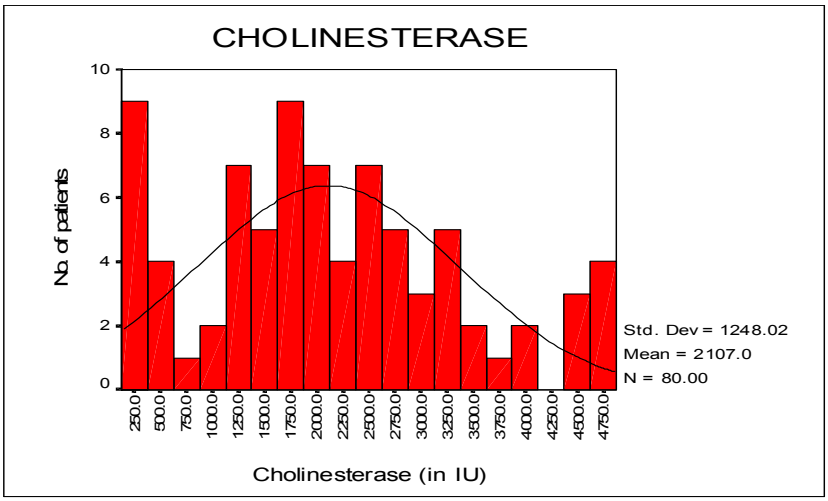
Table-4

Group	A	B	C	D	E
Clear	-	-	-	3	15
Straw colored	47	2	2	-	2
Hemorrhagic	-	6	-	-	-
Purulent	-	-	3	-	-

In this table it is observed that the color of the fluid is straw colored in all the cases in group A where as it is hemorrhagic in most of the cases in group B.

Regarding the lymphocyte count most of the patients in group A had above 60% where as in group D and E count was below 50% in all the cases. For detecting malignant cells in the pleural fluid, the fluid was centrifuged at 1000rpm and the deposit was taken for smear and staining with H&E stain.

The mean cholinesterase level is shown in the figure below:



80 pleural effusion patients were evaluated after exclusion of 4 patients, one with a chylothorax , two patients with more then one etiology and one patient with liver disease. 60 cases fall in group A, B and C. They form the exudative group. Rest of the 20 cases fall in the transudative group i.e. group D and E.

Group	Type	No of cases	%
A	Tb	47	78.34
B	Malignant	8	13.3
C	Parapneumonic	5	8.33
D	CCF	3	15
E	CRF	17	85

In the present study 2 parameters are compared to effectively diagnose whether the pleural fluid aspirated is an exudate or a transudate.

1. Pleural fluid to serum cholinesterase ratio.

2. Pleural fluid to serum protein ratio (Abbreviated Lights criteria).

By using Pleural fluid to Serum Protein ratio (Abbreviated Lights criteria).

Table 5

Exudate / Transudate	***Clinical/Xray/Lab methods***	***Pleural fluid to serum protein ratio***
Exudate	60	65
Transudate	20	15

Using Abbreviated Light's Criteria 5 cases of transudates were misclassified as exudates.

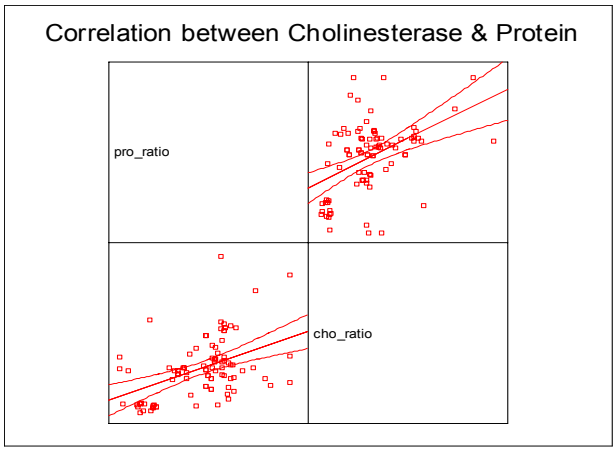
Pleural fluid to Serum Cholinesterase ratio.

Table 6

Exudate / Transudate	*Clinical/Xray/Lab methods*	*Pleural fluid to serum cholinesterase ratio.*
Exudate	60	58
Transudate	20	22

Using Pleural fluid to serum Cholinesterase ratio 58 cases of exudates were correctly classified. About 2 cases of exudates were misclassified as transudates.

Correlation between the Protein and Cholinesterase is shown in the figure below:



Statistical analysis was done by using Pearson's chi-square tests, correlation study by continuity correlation and likelihood ratio by Fisher's exact test. They showed that the sensitivity and specificity using pleural fluid to serum protein ratio was 91.75% and specificity of 75% respectively where as for pleural fluid to serum cholinesterase ratio sensitivity and specificity were 96.7% and 90% respectively.

Comparison with other studies:

Table-7

Parameters	Efficiency		Misclassification	
	Present Study	Manju,Gupta *et al*	Present Study	Manju,Gupta *et al*
Abbreviated Light's Criteria	91.7%	86.36%	8.3%	13.63%
Pleural fluid to serum Cholinesterase ratio	96.7%	98.18%	3.3%	1.82%

This table shows that the present study is almost consistent with Manju Sharma *et al*[3]. cabrer *et al*[16] in his study of 158 cases concluded that

98.7% of the cases were diagnosed correctly using pleural fluid to serum Cholinesterase ratio and concluded that Pleural fluid to serum Cholinesterase ratio is the most accurate criteria in differentiating transudates and exudates.

DISCUSSION

In the past, transudates were separated from exudates by the specific gravity, cell count and the presence or absence of clotting of fluid. In 1972, Light *et al*[1] developed criterion for the diagnostic separation of transudates from exudates. Thereafter it was found that Light's criteria misclassified a large number of effusions which may lead to unwarranted invasive interventions in about 20% to 30% of patients with transudates [20,21]. Therefore some doubts were cast on the universal applicability of Light's criteria. Subsequently, Romero S, Candela A, Martin C, *et al* [15] used modified Light's criteria using new cut-off values. However Vives et al [21] found that change in the classic Light's criteria with different cut-off values offered no advantage in differentiating exudates and transudates. In the present study the diagnostic efficacy of pleural fluid and serum cholinesterase ratio is compared with abbreviated Light's criteria.

Many biochemical parameters like pleural fluid cholesterol, bilirubin, albumin, adenosine deaminase [25, 26] , (MDA) malondialdehyde and their ratios with the serum values have been used to differentiate the types of pleural effusions. Cholesterol is constantly present in pleural effusion and is high level is observed in tubercular effusion.

However, extraordinarily high level of Cholesterol in pleural fluid is independent of it's value in serum [24]. Gupta *et al* [27] studied pleural fluid cholesterol and serum cholesterol ratio in 70 cases of pleural effusion and found that the estimation was not only cost effective but also extremely useful parameter to distinguish transudate from exudate.

Despite these various diagnostic procedures, in 10 to 20% of cases the cause of pleural effusion was uncertain. According to Eduardo *et al* [2] studied about 153 cases and was able to classify about 98.7% of pleura effusions and concluded that pleural fluid to serum cholinesterase ratio was the most accurate criteria for differentiating transudates and exudates.

In our settings usually pleural fluid protein and its ratio with the serum protein is done mainly to differentiate exudates from transudates because of its economic feasibility and its ready availability. Therefore it was thought that it would be prudent if the pleural fluid to serum cholinesterase ratio was compared with pleural fluid to serum protein ratio(Abbreviated Light's criteria).

Using a cut-off value of 0.23 [7] to differentiate exudates from transudates it was found that pleural fluid to serum cholinesterase ratio was significantly higher in exudates than transudates. In the present study the mean pleural fluid cholinesterase in exudates was 2589 and in transudates was 605.3. The difference between the two is statistically significant (P value < 0.001). The mean pleural fluid to serum cholinesterase ratio was 0 .72 in exudates and in transudates is 0.17. According to M.Sharma et al[3] pleural fluid to serum cholinesterase ratio was 0.79 and 0.14 for exudates and transudates respectively in their study. About 86% of cases were in the 3rd to 6th decade of life and male to female ratio being 1 : 0.75. There are a total of 60 cases of exudative effusion of diverse etiology which includes tuberculous effusion, malignant and effusions associated with pneumonia.

There are about 20 cases of transudative effusions out of which 17 cases are due to CRF and the rest are due to CCF.

While comparing pleural effusion using Abbreviated Light's criteria (pleural fluid to serum protein ratio), out of 60 cases of exudative effusion, only 55 cases were identified as exudates, rest of the 5 cases were misclassified as transudates, giving an efficiency ratio of 91.7%.

But while comparing pleural effusion using pleural fluid to serum cholinesterase ratio, out of 60 exudative effusions, 58 cases were correctly identified as exudates, rest of the 2 cases were misclassified, giving an efficiency ratio of 96.7%. The maximum misclassification according to the present study is by applying Abbreviated Light's criteria. When compared to other major studies, the present study is almost consistent with their findings.

The present study has revealed that the pleural fluid to serum cholinesterase ratio is more effective than the pleural fluid to serum protein ratio in diagnosing exudates. The sensitivity of pleural fluid to serum cholinesterase ratio in diagnosing exudative pleural effusion should be evaluated using larger studies.

Therefore is recommended that pleural fluid to serum cholinesterase ratio estimation can be done in all cases of pleural effusions to differentiate between exudates and transudates.

CONCLUSION

1) The results of the present study suggest that pleural fluid to serum cholinesterase ratio is one of the most accurate criteria in classifying pleural effusion.

2) Pleural fluid to serum cholinesterase ratio has more discriminatory capacity than the pleural fluid to serum protein ratio (*Abbreviated lights criteria*) for the same purpose.

3) It is also simple to perform as well as more economical than the tests done for light's criteria.

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MASTER CHART

Sl. No	Name	Age/ Sex	Cholinesterase		Ratio	Protein		Ratio	E/T	Dia.
			Pleural fluid	serum		Pleural fluid	serum			
1	Ramakrisnan	19/m	2847	3711	0.76	5.5	4	1.3	E	TB
2	Tamilarasi	28/f	2442	2858	0.85	4.4	5.2	0.85	E	TB
3	Suresh	39/m	3185	1915	1.66	8.4	6.4	1.3	E	TB
4	Ravi kumar	52/m	2238	3199	0.69	4.2	5	0.84	E	TB
5	Pandian	30/m	1732	1439	1.48	4.8	4.4	1.09	E	TB
6	Dravidachelvan	44/m	3906	7697	0.5	5.3	4.6	1.15	E	TB
7	Srinivasan	50/m	2055	2601	0.79	6.2	7.2	0.86	E	TB
8	Anadavel	33/m	3346	2863	1.16	5.8	6.2	0.94	E	TB
9	Arulmary	30/f	2734	5935	0.46	5.5	4.2	1.3	E	TB
10	Perumal	38/m	2407	3368	0.71	6.2	7.3	0.84	E	TB
11	Priya	27/f	3164	3565	0.88	6.1	7.2	0.83	E	TB
12	Sasikala	14/f	2731	3684	0.74	3.3	6.4	0.5	E	TB
13	Ganesan	40/m	4637	2493	1.86	5.3	6	0.88	E	CA
14	Jayashanker	30/m	3975	5458	0.72	5.7	6.7	0.85	E	TB
15	Sarojamma	60/m	2409	7450	0.32	4.4	6.2	0.7	E	TB
16	Sasikumar	29/m	1868	3117	0.59	4.1	4.8	0.85	E	TB
17	Prabhu	23/m	4703	9293	0.5	4.9	5.2	0.94	E	TB
18	Balasubramani	22/m	4791	6570	0.73	3.8	4.6	0.83	E	CA
19	Adhi	40/m	3371	8250	0.41	3.9	4.2	0.93	E	CA
20	Rajathi	55/f	1185	7935	0.15	2.5	5.4	0.46	T	CRF
21	Vadival	45/m	2204	5068	0.43	4.7	4	1.18	E	TB
22	Pandurangan	65/m	300	2349	0.13	2.2	5.6	0.39	T	CCF
23	Meenakshmi	17f	4584	6503	0.7	4.5	5	0.28	E	TB
24	Shanthi	43/f	2265	3057	0.74	3.8	4.2	0.9	E	TB
25	Mesakrajan	18/m	1231	1075	1.14	4.6	5.2	0.88	E	TB
26	Tulasi	25/f	2191	2092	1.04	5.2	5.8	0.9	E	TB
27	Sivakami	55/f	4511	4180	1.07	6.2	6.8	0.91	E	TB
28	Gandhi	41/m	626	2832	0.22	2.1	4.9	0.42	T	CCF
29	Kannan	58/m	4430	7912	0.55	4.7	5.3	0.89	E	TB
30	Minnal	38/f	1171	4135	0.28	4	4.3	0.93	E	TB
31	Panchalai	50/f	1654	4918	0.33	4.4	4.8	0.92	E	TB
32	Sher bahadur	55/m	2562	5022	0.51	5.1	6.2	0.82	E	TB
33	Palayam	60/m	3183	7964	0.39	4.1	5	0.82	E	CA
34	Shanthi	32/f	2656	4915	0.54	4.6	5.1	0.9	E	TB
35	Prabhakar	36/f	2408	3617	0.66	6.1	6.5	0.94	E	TB
36	Rani	29/f	3099	7381	0.42	3.9	4.9	0.79	E	PARA
37	Gopinath	25/f	3449	5043	0.68	5.9	6.3	0.93	E	TB
38	Vembuliammal	80/f	4636	7807	0.59	6	5.9	1.01	E	TB
39	Arulmary	30/f	2501	3780	0.66	5.7	6	0.95	E	TB
40	Devanathan	54/m	3412	4080	0.84	4.7	6.4	0.73	E	CA
41	Bhadrachalam	56/m	1114	1775	0.63	6.2	6.7	1.08	E	TB
42	Elango	33/m	2927	4210	0.69	4.9	5.5	0.89	E	PARA
43	Gyananavel	28/m	2021	3117	0.65	3.9	5	0.78	E	TB
44	Fathima	34/f	1914	3074	0.62	4.7	5.3	0.89	E	TB
45	Raghu	22/m	1980	1839	1.07	5.8	6	0.97	E	TB
46	Palani	42/m	1904	1787	1.06	5.2	5.9	0.88	E	PARA

47	Kaliammal	49/f	1391	2680	0.52	5	5.2	0.96	E	TB
48	Raji	33/f	1324	3653	0.36	5.4	5.6	0.96	E	TB
49	Vasantha	43/f	1750	2756	0.63	4.3	5.2	0.83	E	TB
50	Govindasamy	62/m	2954	2703	1.09	5.9	6.3	0.94	E	TB
51	Anbu	18/m	1820	1636	1.11	5	5.5	0.9	E	PARA
52	Kumar	32/m	2409	2452	0.98	4.7	5.9	0.79	E	TB
53	Vasanthi	33/f	1661	1782	0.93	5.2	5.8	0.89	E	TB
54	Devaraj	52/m	300	1299	0.23	2	5	0.4	T	CRF
55	Padmalakshmi	54/f	585	2598	0.22	1.4	4.8	0.29	T	CRF
56	Arumugam	59/m	1578	2900	0.54	5.8	7.2	0.8	E	CA
57	Indra	31/f	450	2006	0.22	2.2	5.6	0.39	T	CRF
58	kesavan	39/m	3804	4900	0.78	4.8	6.9	0.69	E	CA
59	Sulochona	52/f	280	1345	0.21	2.7	6.1	0.44	T	CRF
60	Devi	42/f	310	1600	0.19	2.3	6	0.38	T	CRF
61	Periyasamy	57/m	225	1046	0.21	2	5.4	0.37	T	CRF
62	Murugesan	39/m	330	1863	0.18	2.6	5.5	0.47	T	CRF
63	Kalpana	24/f	425	2153	0.2	2.7	5.8	0.47	T	CCF
64	Mohan	48/m	250	1700	0.15	2.2	5.3	0.41	T	CRF
65	Venu	50/m	380	1890	0.2	3	6.5	0.46	T	CRF
66	Kavitha	28/f	200	1008	0.19	2.9	5.9	0.49	T	CRF
67	Munisamy	61/m	254	1238	0.21	3.2	6.6	0.48	T	CRF
68	Ravi	47/m	670	4989	0.13	4.4	7	0.42	E	TB
69	Rani	52/f	1852	3108	0.59	4.2	6.9	0.6	E	TB
70	Shanthi	29/f	1250	2415	0.51	4.6	6.8	0.67	E	TB
71	Rajendiran	45/m	1666	2896	0.57	4	5.9	0.48	E	TB
72	Murugantham	30/m	1113	1780	0.62	3.8	6.6	0.57	T	CRF
73	Rafiq	21/m	1314	2074	0.6	4.7	5.8	0.79	E	TB
74	Sujatha	29/f	608	4623	0.13	2.7	6.1	0.44	T	CRF
75	Vidya	35/f	1962	3144	0.62	4.4	6.3	0.66	T	CRF
76	Ramamuruthy	41/m	1896	2968	0.63	3.9	5.9	0.65	E	PARA
77	Chinnaponnu	25/f	1413	2880	0.49	4	6.7	0.59	E	CA
78	Rajalakshmi	19/f	1510	2545	0.19	4.7	7.1	0.66	T	CRF
79	Velu	29/m	1456	2603	0.15	3.9	6.2	0.62	T	CRF
80	Brinda	40/f	1319	2213	0.22	3.8	6.1	0.62	T	CRF