The Value of Measurment of Bilirubin in Pleural Fluid in the Differentiation between Exudative and Transudative Pleual Effusion

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Abstract

Background: The pleural effusion is a common clinical problem which can be exudative or transudative in nature due to variety of causes. Measurement of biochemical markers in the pleural fluid is an important measure to distinguish exudative from transudative effusion.

Aim of the Study: Is to determine the value of pleural fluid to serum bilirubin ratio to differentiate between transudative and exudative pleural effusion.

Patients and Method: Thirty-five patients enrolled in this study, they admitted to medical ward in Baghdad teaching hospital and submitted to clinical, radiological, ultrasonographic examination proved to have pleural effusion from December 2018 to July 2019. Samples of blood and pleural fluid aspirated in the medical ward, analyzedafter 30-60 minute of aspiration. The sample of some cases were analyzed in private laboratories. Serum protein, total serum bilirubin and other investigation according to the condition of the patient like blood urea, serum creatinine, liver function test, sputum of AFB. Pleural fluid analyzed from protein, total bilirubin, cell count, differential and cytology .Pleural biopsy was done to patients suspected to have malignant or tuberculous effusion and grouped at the end of their hospitalization into exudative and transudative effusion according to specific etiology, clinical examination, radiological finding, and then measure the pleural fluid to serum protein ratio, pleural fluid to serum bilirubin for each patient. We depended on pleural fluid to serum protein ratio to compare with pleural fluid to serum bilirubin ratio for differentiation between exudative and transudative pleural fluid.

Results: Twenty two patients with exudative effusion and thirteen patients were having transudate according to pleural fluid to serum protein (Light 's criteria). P-value= 0.002, Fisher. Exact test= 0.001. Twenty three patients with exudative effusion and twelve patients with transudative effusion according to pleural fluid to serum bilirubin ratio. P value= 0.37, Fisher. Exact test = 0.45. Pf/s ratio can increase the sensitivity and specificity of Light 's criteria.

Conclusions: Pleural fluid to serum bilirubin ratio can't replace Light's criteria in differentiation between exudates and transudate pleural effusion. Pf/s bilirubin ratio is a reliable test can easily perform but not common use in our hospital because of its costy and not familial use in our laboratories.

Keywords: *Bilirubin; Pleural fluid; exudative; Transudative pleural effusion.*

Introduction

The pleural lining is a serious membrane covering the lung parenchyma, chest wall, diaphragm, and mediastinum. The pleural membrane covering the surface of the lung is known as visceral pleura, whereas the parietal pleura cover the remaining mentioned structures. In between the visceral and parietal pleura is the pleural space, a potential space that contain a thin layer of fluid (approximately 0.15 ml/kg/pleural space) usually around 10 ml in each pleural space. The parietal pleura secrete approximately 2400 ml of fluid daily, which is reabsorbed by the visceral pleura[(1).]A

pleural effusion is present when there is an excess quantity of fluid in the pleural space. [2].Pleural fluid accumulates when pleural fluid formation exceeds pleural fluid absorption; fluid enters the pleural space from the capillaries in the parietal pleura and is removed via lymphatic situated in the visceral pleura. Fluid can also enter the pleural space from the interstitial space 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 pleural effusion may develop when there is excess pleural fluid or peritoneal fluid or when there is decreased fluid removal by the lymphatics. [1]. The distinction between a transudative and exudative pleural fluid is an important step in the diagnosis of the cause of the pleural effusion by using various parameters, like Light's criteria (1972) p/f is exudate if one or more of the following criteria are met [3,4]. (Pleural fluid protein/serum protein > 0.5, Pleural fluid LDH/serum LDH >0.6,Pleural fluid LDH > two-third of upper limit of normal serum LDH)A pleural fluid to serum bilirubin ratio of more than 0.6 was suggested as an alternative to Light's criteria for distinguish exudates from transudate pleural fliud.Dyspnea is the most common symptom associated with pleural effusion, it related more to distortion of the diaphragm chest wall during respiration than to hypoxemia. In many patients drainage of pleural fluid alleviate symptoms despite limited improvement in gas exchange.Chest symptoms of pleural effusion include mild, non productive cough or chest pain . Other symptoms may suggest the etiology of pleural effusion e.g. more severe cough or production of purulent sputum suggest an underlying pneumonia or endobronchial lesion [5].Constant chest wall pain may reflect chest wall invasion by bronchogenic carcinoma or malignant mesothelioma.Pleurtic chest pain suggests either pulmonary embolism or an inflammatory pleural process.Systemic toxicity evidence by fever, weight loss and inanition suggest empyma[6].PHYSICAL EXAMINATION: Restriction in the chest expansion of one side or both sides, Trachea and apex beat may be displaced away from the effusion if it is large, Movement of affected side may be reduced, Percussion note is stony dull, Vocal fremitus and resonance are reduced, Breath sounds are reduced.Initial testing focuses on confirming that a pleural effusion is present. A chest x-ray study is the typical starting point. In the upright anteroposterior view, a small effusion may show up as blunting of the costophrenic angle. Larger effusions show a meniscus sign at the air fluid border. Lateral decubitus views help estimate the size of the effusion [7].Ultrasound can detect as little as 5 to 50 ml of fluid. It is also helpful in locating pockets of fluid and guiding thoracentesis for small effusions. Computed tomography, which is very sensitive, can differentiate pleural fluid from pleural thickening and focal masses[7] . Thoracocentesis allows evaluation of any undiagnosed pleural effusion. Note that not all effusions require diagnostic thoracentesis. If the cause is apparent from the clinical presentation (e.g. CHF), observation may be appropriate [5]. In general, parapneumonic effusions require thoracentesis to confirm diagnosis and assess the need for chest tube placement.

Patients and Method

Thirty-five patients enrolled in this study were admitted to medical ward of Baghdad teaching hospital and submitted to clinical examination and investigation, proved to have pleural effusion in the period from December 2018 to July 2019.Samples of blood and pleural fluid that were aspirate in the medical ward, analyzed after 30-60 minute of aspiration. Some of the cases analysis of their samples done in private laboratories. Serum analysis for protein concentration, total bilirubin and other investigation according to the condition of the patient like blood urea, serum creatinine, liver function, sputum for AFB and cytology. Pleural fluid analyzed for protein, total bilirubin, cell count, differential and cytology. Then calculate the pleural fluid to serum bilirubin for each patient and compare with pleural fluid to serum protein which measured also for each patient. The procedure for measuring protein in pleural fluid is called ZeilfaBiurate method, by taking 1.4 ml distal water mix with 0.1 fluid and 2.5 ml biurate, wait for 10 minuteat room temperature then read the result by spectrophotometer at 540mm. The method for determination of bilirubin concentration in plasma (serum) which is the same use for measurement of total bilirubin in pleural fluid are divided into the method using:-Diazo reagent & DPD (dichlorobenzenediazonium salt) & Bilirubin oxidaze & Direct spectrophotometric determination of bilirubin. The procedure which use in this research is the first one was Van den Berg's reaction with diazobenzenesulfonic acid from 1913. Method have been developed for the determination of total bilirubin (conjugate and unconjugated) or conjugated bilirubin alone. The proportion of unconjugated bilirubin is calculated as difference between total and conjugated bilirubin should previously be separated from albumin by use of an accelerator (methanol, sodium acetate

diphylline, sodium benzoate-sodium acetate-caffeine or detergent, e.g. Brij35) those conjugated bilirubin reacts directly in the reaction of diazotization. Bilirubin diazotization is generally performed by use of mixture of sulfanilic acid and sodium nitrite, however, diazonium salts of 2,5-dichlorophenyl or 2,4-dichloroaniline have recently also been introduced. The determination of bilirubin concentration by use of these method based on the formation of azobilirubin, which acts as an indicator. Is pink in acidic or neutral medium, and blue-green in alkaline medium.

Statical Analysis: Chi square were used to study the variations in the ratio of pf/s protein ratio of different diseases and pf/s bilirubin ratio of different diseases. Also using F. E.t . F-test was used to study the variations of total serum protein, pleural fluid protein, total serum bilirubin and pleural fluid bilirubin.

Results

Table (1): Classification of pleural effusion according to causes.

Diagnosis	No . of patients	Percentage
Pulmonary TB	11	31.42%
Bronchogenic carcinoma	6	17. 14%
Pulmonary Metastasis	5	14. 28%
Pneumonia	2	5 .71%
Undiagnosed		
HF	3	8.57%
RF	6	17.14%
Total	35	100%

Table (1) shows the number of patients with pleural effusion and the causes of their pleural effusion. The number of patient with tuberculous pleural effusion were 11 (31.43%), while those with bronchogenic carcinoma were six patients (17.14%), patients with pulmonary metastasis were five patients (14.28%) of patients with RF were 6 (17.14%) while those patients with heart failure were 3 (8.57%) and those cases undiagnosed were 3 (8.57%).

Table (2): Distribution of cases according to the age

Age (Year)	Number of patients	Percentage
10 - 19	2	5.71%
20 – 29	6	17.14%
30 – 39	3	8.57%
40 – 49	5	14,28%
50 – 59	4	11.42%
60 – 69	10	28.57%
70 - 79	5	14.28%

Table (3):Classification of pl –effusion into exudate & transudat by f/s Brlirubin ratio .

Type of fluid	Value of pf/s B.R	No .of patients	Percentage
Exudate	≥0.6	23	65.71%
Transudate	< 0.6	12	34.28%
Total		**35 Case**	

Table 4: Classification of pl –effusion into exudates & transudate by F/s Protein ratio .

Type of fluid	Value of pf/s pro.R	No .of patients	Percentage
Exudate	≥0.5	22	62.28%
Transudate	< 0.5	13	37.71%
Total		**35 Case**	

Table (5): Distribution of diseases according to ratio of protein in pleural fluid to total serum protein

Diseases	Ratio			
	≥0.5		<0.5	
	No.	%	No.	%
Pulmonary TB	9	81.8	2	18.2
Lung CA	5	83.3	1	
Heart failure	0	0.0	3	100.0
Secondary metastasis	5	100.0	0	0.0
Undiagnosed	1	50.0	1	50.0
Chronic renal failure	0	0.0	6	100.0

Diseases	Ratio			
	≥0.5		<0.5	
	No.	%	No.	%
Pneumonia	1	50.0	1	50.0
Total	21	60.0	14	40.0

c^2=20.5, d.f=6, p=0.002,Fisher `s Exact test=0.001

Table (6): Distribution of diseases according to pleural fluid to total serum bilirubin ratio.

Diseases	≥0.6		<0.6	
	No.	%	No.	%
Pulmonary TB	6	54.5	5	45.5
CA. lung	3	50.0	3	50.0
Heart failure	3	100.0	0	0.0
Secondary metastasis	4	80.0	1	20.0
Undiagnosed	1	50.0	1	50.0
Chronic renal failure	2	33.3	4	66.7
Pneumonia	2	100.0	0	0.0
Total	**21**	**60.0**	**14**	**40.0**

c^2=6.4,d.f=6, p=0.37,Fisher`s exact test=0.45.

Table (1) shows the patients' distribution number according to age which shows that the most common group ranged from (60 - 69) were 10 (28. 57), the second group ranged from (20 - 29) were 6 (17. 14%) while those group range between (40 - 49) were5 (14. 28%) equal to those group ranged between (70 - 79) were 5 (14. 28) and the least number those group (10-19) were 2 (5.71%). To determine whether these cases in my research have exudates pleural effusion or transudate is 0 .6 fluid to serum bilirubin ratio as show in table (3). There are 23 (65.71%) patients with exudative type their f/s ratio of Bilirubin is equal to or more than 0. 6 while those with transudative type are 12 (34.28%) patients their f/s ratio less than 0.6 Table (4) show the cut off point for f/s protein ratio is 0.5. The number of patients whom their f/s equal/or more than 0. 5 are exudative pleural effusion Their number are 22 (62. 28) while those with transudative type have f/s ratio less than 0.5 their number are 13 (37, 71) .

Table (5) shows the distribution of diseases according to ratio of protein in Pleural fluid to total serum protein, Those patient with pulmonary TB occupied We larger number there are nine patients (81.8) with a ratio more than or equal 0 0. 5 and two patients (182) with a ratio less than 0. 5. In bronchogenic Carcinoma only one case misdiagnosed with transudate pleural effusion. p.value = 0.002 . Fisher 's Exact test = 0.001 Table (6) show the distribution of diseases according to level of bilirubin In pleural fluid to total serum bilirubin only six patients of pulmonary tuberculosis catch that have exudative pleural effusion and five of them diagnosed wrongly had transudate pleural effusion when depend on Pf/s bilirubin ratio.also for bronchogenic carcinoma I catch three patients with exudative pleural effusion While other diagnosed wrongly with transudate pleural effusion. p.value = 0.37, fisher 's exact test = 0.45 If the p- value or F. E. T less than 0.05 were consider as significant.

Discussion

The use of several chemical tests to separates transudates from exudates is recommended as a useful first step in determining the cause of pleural effusion. In 1972 Light et al used pleural and serum levels of proteins and LDH to establish criteria for segregation transudates from exudates with a sensitivity and specificity both near 100 percent (19, 20, 21) this high diagnostic accuracy together with a relatively low cost made the criteria of Light et al the gold standard for initial categorization of

pleural effusions. Recently, however, several prospective studies (22, 23) were unable to reproduce the excellent results obtained by Light *etal.,*as show in this study pf/s bilirubin, Bilirubin of molecular Weight of 584 behaves similar to high molecules weight protein with respect to its concentration distribution between serum and pleural fluid (24), Such behavior may be attributed to structural and electrical properties of bilirubin, but it seems that it can not be based sound biochemical reasoning other than the plausible mechanism of protein binding.

This characteristic may explain why the bilirubin and protein ratios concentration in exudative effusion is higher than transudate which reflect serum level in circumstances that increase permeability of pleural membrane, but the biochemical analysis of bilirubin affect by light, duration between taking the sample and analysis When the duration is long or when the sample expose to light, there is more chance for wrong result so when sample of pleural fluid of patient send to be analyzed outside the hospital should protected from light and kept with preserve material (anticoagulant). Also other drawback of this method that there 's no specific method in determination of bilirubin in pleural fluid although there' s different in consistency between serum and pleural fluid. In this research the Pf/s bilirubin ratio was not sufficient alone to differentiate between exudates and transudate pleural fluid in comparison with Pf/s protein ratio (light's criteria). Some vale 200 sensitivity of the test and other give low Sensitivity of the test for e.g. the sensitivity of pulmonary tuberculosis effusion is 94% bronehonic carcinoma as 92% and for parapneumonic effusion is 58%.

Also by stastical analysis using p -value & Fisher's Exact test show no significant result according to Pf/s bilirubin ratio but it give Significant result with Pf/s protein ratio. There is similar study done in clinical biochemistry department in William Harvey hospital using multiple biochemical parameters for differentiation between exudates and transudate pleural fluid. The recommendation of this study shows that the bilirubin is not useful [(8)]. Other Study done in department of chest disease, Cumhuriyet university medical School, Sivas Turkey show that can use different biochemical parameter like measure Pleural fluid and serum levels of albumin, protein, LDH, cholesterol and bilirubin of 381 patient with pleural effusion[(25)]. Also there's thesis prepared by Dr. Hashim Mahdi for degree of fellowship of Iraqi board of medical specialist in internal medicine. These two studies give result reverse to my study that Pf/s bilirubin play a role in distinguish between exudates and transudate and it can increase the sensitivity and Specificity of Light's criteria.Note; there is no facility in the hospital lab. for other biochemical test like LDH, cholesterol, ADA also PH of fluid and because of limited material that use for measure concentration of bilirubin in serum or in fluid,the head master of biochemical department accept one case per week of my research to measure the bilirubin in fluid that's why I deal with limited number of patient and the of my research need large group of patients to asses and reach to good result.

Conclusions

Pleural fluid to serum bilirubin ratio can't replace Light's criteria in differentiation between exudates and transudate pleural effusion. Pf/s bilirubin ratio is a reliable test can easily perform but not common use in our hospital because of its costy and not familial use in our laboratories.

Conflict of Interest: None

Source of Findings: None

Ethical Clearance: None

References

1. Richard W.Light, Kasper DL, Fausietal. Disorder of the pleura in Harrison's principles of internal Medicine 2003, 1565.
2. Washington manual pulmonary medicine subspecially consult, the 1st edition 23 160, 161.
3. Bartolome R.Celli, Slovis BS, Bigham KL etal. Diseases of pleura in Cecil textbook of medicine, 21 ed. vol. 1, 2000: 457- 460.
4. Heffner JE, Brown LK, Barbari CA. Diagnostic value of tests that discriminate between exudative and transudative pleural effusion chest 1997, 11 970 – 980.
5. Heffner JE Brown LK, Barbieri CDeleo JM: Pleural fluid chemical analysis in parapneumonic effusions. Mea-analysis AM J Respir Crit Care Med 1995 Jun; 151 (6): I700 – 8.
6. Kumar & Clark: clinical medicine GE 2006, 14,: 86, 894.
7. Lawrence M. tierney J et al, Current medical diagnosis and treatment 45th, 2006, p 302.