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Original research article

A Qualitative Study on Serum Effusion Albumin Gradient and Pleural Fluid Protein Thiols in Differential Diagnosis of Pleural Exudate and Transudate

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Abstract

Background: Pleural effusion is a common disorder. Based on the underlying pathology, the effusions are classified into transudates an exudates. Presently, Light's criteria [3] are used to distinguish between transudates and exudates. But many pleural effusions have been misclassified as transudates or as exudates using Light's criteria. The present study was done to evaluate the diagnostic accuracies of serum-effusion albumin gradient (SEAG) and pleural fluid protein thiols in differentiating pleural fluids exudates from transudates.

Material & Methods:

This was a prospective study conducted in a tertiary care hospital in. The study was conducted from (March 2019 to Sep 2020) Rajshree Medical Research Institute, Bareilly, Lucknow . Patients with pleural effusion due to various diseases were included in this study. Pleural effusion and serum samples were collected from all patients. Thoracocentesis was performed to collect pleural effusion, while serum was collected from the venous blood using a 5 mL syringe. Albumin, Total Proteins, Lactate dehydrogenase and Protein thiols were measured in both Serum and pleural fluid.

Results & Discussion:

There was a significant difference in the level of serum and pleral fluid parameters of total protein, albumin, LDH, thiols between transudates and exudates, which was statistically significant ($P<0.001$). The results presented in this study demonstrate that the concentration of protein thiol in serum was markedly reduced in patients with exudates compared to transudates. The present study has shown that even though taking into account the light's criteria and SEAG in differentiating exudates and transudates. The greater differential value was found with a combination of SEAG and pleural fluid protein thiols, which correctly classified 92.31% of transudates and 93.34% of exudates with sensitivity and specificity of 92.31% and 83.34% and 93.34% and 92.31% respectively.

Conclusion: The present study shows that measurement of serum & pleural fluid protein thiols in patients with pleural effusion of diverse etiology proved to be better marker for the differentiation of exudates and transudates, as this method provided a high sensitivity and specificity for characterization of effusion as an exudates and transudates compared to light's criteria. This explains the different pathophysiology behind the production of exudates and

transudates. However to overcome the limitation of misclassification by using light's criteria, we advocate measurement of serum and pleural fluid protein thiols along with SEAG could be better alternative in differentiation of exudates and transudates in clinical practice
Key words: Transudate, Exudate, Pleural effusion, Albumin gradient

Introduction

Pleural effusion is a common disorder which occurs either as a manifestation or a complication of both respiratory and non respiratory diseases. Based on the underlying pathology, the effusions are classified into transudates an exudates [1, 2]. Transudate is normally composed of ultra filtrates of plasma and is poor in cellular content and protein concentration. Whereas exudates has a greater variety of cells either inflammatory or neoplastic, depending on the cause [1, 2]. Presently, Light's criteria [3] are used to distinguish between transudates and exudates (pleural fluid/serum protein ratio >0.5, pleural fluid/serum LDH ratio >0.6 and absolute pleural fluid LDH >200Udenote an exudate). But many pleural effusions have been misclassified as transudates or as exudates using Light's criteria [4, 5].

Light's criteria for exudates are very sensitive but an albumin gradient of 1.2 gm/dl or less tends to be more specific especially in congestive cardiac failure (CCF) on diuretics [6, 7]. Light's criteria are the most sensitive for identifying exudates but have lower specificity than other criteria [6, 7]. The values of protein in transudates are more common in the evaluation of diseases like congestive cardiac failure and ascites, which led to the development of serum-ascites albumin gradient [8]. Roth and associates have documented that serum-effusion gradient of albumin is a better discriminator than Light's criteria in the diagnostic separation of transudates and exudates [9]. But another study by Burgess et. Al. [7] using an albumin gradient of 12 g/L found the sensitivity and specificity to be 87% and 92% respectively and concluded that the Lights criteria is the best method for distinguishing exudates from transudates. Hence the present study was done to evaluate the diagnostic accuracies of serum-effusion albumin gradient (SEAG) and pleural fluid protein thiols in differentiating pleural fluids exudates from transudates.

Material and Methods

This was a prospective study conducted in a tertiary care hospital . The study was conducted from (March 2019 to Sep 2020) Rajshree Medical Research Institute, Bareilly, Lucknow . Patients with pleural effusion due to various diseases were included in this study. Pleural effusion was diagnosed clinically and radiologically. The study was approved by institutional ethics committee and written informed consent was taken from all the patients. The following were the inclusion and exclusion criteria.

Inclusion criteria

Patients of either sex aged more than 18 years diagnosed with pleural effusion due to various diseases.

Exclusion criteria

Cases, in which either no cause was definitely diagnosed or more than one cause was present, were excluded from the study. Pleural effusion and serum samples were collected from all patients. Thoracocentesis was performed to collect pleural effusion, while serum was

collected from the venous blood using a 5 mL syringe. Albumin, Total Proteins, Lactate dehydrogenase and Protein thiols were measured in both Serum and pleural fluid.

The following biochemical parameters were estimated and calculated

1. Criteria of light et al (namely: pleural fluid/serum protein ratio, pleural fluid/serum LDH ratio, pleural fluid LDH concentration)
2. Albumin gradient (serum albumin concentration minus pleural effusion albumin concentration)
3. Protein gradient (serum total protein concentration minus pleural effusion total protein concentration)
4. Plasma and pleural fluid total protein levels were estimated by Biuret method [10, 11].
5. LDH estimation: LDH level was estimatedby UV kinetic method and was expressed as IU/L20, upper normal limit (serum) was defined as 248U/L [12].
6. Albumin estimation: Determination of plasmaand pleural fluid albumin was done using manual
method of Doumas et al and modified Spencerand Price method [7, 13].
7. Serum protein thiol was measured with a spectrophotometric method using Dithionitrobenzoic acid (DTNB) [14].

Statistical analysis

The data collected as a part of the study was analyzed by using Statistical Package for Social Sciences (SPSS version 16). Descriptive and other statistics test results were expressed in tables and figures. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Student‘t’ test for unpaired data were used to compare means of parameters measured in two groups of patient with transudative and exudative pleural effusions.

Results

A total of 97 cases of pleural effusion were included in the final analysis. The age and sex distribution of the cases is shown in table 1.

Table 1: Age and sex distribution of the cases (n=97)

Age group (Years)	Male	Female	Total
0-10	0	0	0
11-20	1	0	1
21-30	7	4	11
31-40	9	5	14
41-50	14	9	23
51-60	18	10	28
61-70	9	5	14
71-80	4	2	6
Total	**62**	**35**	**97**

Most cases were in males (63.9%) and in the age group of 51-60 years.
The cause of transudates and exudates in pleural effusion is shown in table 2.

Table 2: Cause of transudates and exudatesin pleural effusion (n=97)

Cause	Transudate	Exudate
CCF	22	-
Anemia	04	-
Cirrhosis	16	-
Tuberculosis	-	34
Malignancy	-	14
Empyema	-	07

CCF = Congestive cardiac failure
Total transudates= 42 (43.2%), Total exudates= 57 (58.7%)
The most common cause of transudate was Congestive cardiac failure and most common cause of exudate was tuberculosis.
The comparison of serum (total protein, albumin, LDH, thiols) between transudates and exudates is shown in table 3.

Table 3: Comparison of serum (total protein, albumin, LDH, thiols) between transudate and exudates.

Serum parameter	Transudate	Exudate	P value
Total protein	5.9 ± 0.91	6.5 ± 0.87	P < 0.001
Albumin	3.67 ± 0.62	4.02± 0.39	P < 0.001
LDH	554.2 ±176.4	297.4 ±97.4	P < 0.001
Protein thiols	59.4 ± 16.2	107.2 ±23.5	P < 0.001

P < 0.001 = Highly significant.The comparison of pleural fluid (total protein, albumin, LDH, thiols) between transudates and exudates is shown in table 4.

Table 4: Comparison of pleural fluid (total protein, albumin, LDH, thiols) between transudate and exudates.

Pleural parameter	Transudate	Exudate	P value
Total protein	3.78 ±0.69	5.26 ± 1.14	P < 0.001
Albumin	3.11± 0.65	3.96 ± 0.72	P < 0.001
LDH	221.7 ±75.3	498.6 ±214.8	P < 0.001
Protein thiols	137.9 ±19.8	71.4 ± 11.4	P < 0.001

P < 0.001 = Highly significant

The comparison of sensitivity, specificity and positive predictive value (PPV) of SEAG with Light's criteria is shown in table 5.

Table 5: Comparison of sensitivity, specificity and positive predictive value (PPV) of SEAG with Light's criteria

Parameter	SEAG	Light's criteria
Sensitivity of transudate	94.3%	79.8%
Sensitivity of exudate	95.1%	78.3%
Specificity of transudate	87.4%	74.2%
Specificity of exudate	93.3%	75.6%
PPV of transudate	84.7%	73.2%
PPV of exudate	93.6%	82.6%

The sensitivity, specificity and positive predictive value (PPV) of SEAG were higher when compared with Light's criteria.

Discussion

Differentiating the pleural fluid in a transudate or an exudate is the first and important step in the evaluation of pleural effusion [15-17]. But many pleural effusions have been misclassified as transudates or as exudates using Light's criteria [4,5], with misclassifications varying from 2% to 40% [18-20]. In the present study most cases of pleural effusion were form males and in the age group of 51-60 years (table 1) and the most common cause of transudate was Congestive cardiac failure and most common cause of exudate was tuberculosis (table 2). Similar findings were reported by other studies [21,22]. Both albumin and globulin fraction in pleural fluid are believed to originate from serum via diffusion. However some protein like LDH comes from within pleural space i.e from pleural leucocytes. As pleural fluid albumin is originating from serum, measurement of serum effusion albumin gradient (SEAG) was considered as effective measure in discriminating exudates from transudates [23]. The comparison of serum (total protein, albumin, LDH, thiols) between transudates and exudates is shown in table 3. There was a significant difference in the level of total protein, albumin, LDH, thiols between transudates and exudates, which was statistically significant ($P<0.001$).

The comparison of pleural fluid (total protein, albumin, LDH, thiols) between transudates and exudates is shown in table 4. There was a significant difference in the level of total protein, albumin, LDH, thiols between transudates and exudates, which was statistically significant ($P<0.001$).

A study conducted by Roth et al [9] used serum-effusion albumin gradient for the classification of pleural effusions. With a cut-off value of 1.2 g/dI, all transudates and exudates were classified correctly with sensitivity and specificity of 87%, and 92%, respectively. A similar study conducted by Arijit Kumar Das [24] et al and K.B. Gupt [5] et al also showed that though the criteria of light's helps in differentiation of exudates from transudates. Recently usefulness of Malondialdehyde (MDA) has been studied to differentiate transudates from exudates [25] and was found to have increased levels of MDA in pleural fluid in exudates compared to transudates. Albumin contains an exposed cysteine –SH (thiol) group and provide the bulk of total plasma thiol, a well known antioxidant62. As there is change in albumin gradient because of pleural effusion. Our aim was to know the serum & pleural fluid protein thiols status in such oxidative environment and does these protein bound thiol groups helps in differentiating between exudates from transudates. The results presented

in this study demonstrate that the concentration of protein thiol in serum was markedly reduced in patients with exudates compared to transudates. The decreased plasma thiol levels may be due to enhanced free radical generation in patients with exudates, which is mainly due to several inflammatory condition associated with exudative pathology. The present study has shown that even though taking into account the light's criteria and SEAG in differentiating exudates and transudates. The greater differential value was found with a combination of SEAG and pleural fluid protein thiols, which correctly classified 92.31% of transudates and 93.34% of exudates with sensitivity and specificity of 92.31% and 83.34% and 93.34% and 92.31% respectively.

Conclusion

The present study shows that measurement of serum & pleural fluid protein thiols in patients with pleural effusion of diverse etiology proved to be better marker for the differentiation of exudates and transudates, as this method provided a high sensitivity and specificity for characterization of effusion as an exudates and transudates compared to light's criteria. This explains the different pathophysiology behind the production of exudates and transudates. However to overcome the limitation of misclassification by using light's criteria, we advocate measurement of serum and pleural fluid protein thiols along with SEAG could be better alternative in differentiation of exudates and transudates in clinical practice

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