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To evaluate role of interleukin-8 in differentiating transudative and exudative pleural effusion

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Abstract:

Introduction: Pleural effusion appears in approximately 40% of patients with pneumonia. Given that microbiology results are often negative, its diagnosis is frequently based on clinical criteria. Our study consisted of 240 patients, divided into infectious (n = 28), tuberculous (n = 48), paraneoplastic (n = 58), miscellaneous exudates (n = 48), and transudates (n = 58). Interleukin (IL)-6, IL-8, and IL-1β were measured in the pleural fluid and serum of all patients, as well as the different cell populations in the pleural fluid.

Material and Method: This is a tertiary care hospital based cross sectional study which included 60 patients with pleural effusion. Patients were included in the study after getting informed consent from the patient and patient's attender. Also, an ethical committee approval was obtained. Clinical history was elicited and physical examination was performed. Pleural effusion was confirmed based on the chest X-ray finding. USG thorax/CT thorax was taken if the effusion was lobulated or minimal. After diagnosing pleural effusion, they were further divided into two categories, namely transudate and exudate based on Light's criteria. Pleural fluid was obtained by diagnostic pleural fluid aspiration (thoracentesis) after informed consent.

Results: Based on Light's criteria, of the 60 patients, 35 patients had exudative pleural effusion and 25 patients had transudate effusion. In the transudates subgroup, the maximum number of patients were between 41 to 60 years, whereas in exudates, the bulk of the study population consisted of the age group 21 to 40 years and the difference in the mean age between these two subgroups was found to be insignificant. In both the subgroups, male patients contributed the bulk of the study population. Among the transudates subgroup, the maximum numbers (43%) were due to heart failure, followed by chronic kidney disease and cirrhosis of liver. 39% of exudates were due to tuberculosis followed by heart failure, malignancy, abscess, Para pneumonic effusion and abdominal causes.

Conclusion: Although the classical parameters have a better specificity and sensitivity than IL-8, its value is important because it is only one parameter for determination versus three biochemical parameters (LDH, glucose and pH). IL-8 may be used as an alternative marker for the complication of Para pneumonic effusion but its diagnostic value needs further analysis.

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Keywords: Interleukin 8; Pleural fluid, Pleural effusion, Exudative effusion, Transudate effusion, Empyema.

Introduction

Pleural effusion is a common complication in numerous diseases, and the differential diagnosis is occasionally difficult to obtain without using invasive procedures.[1] Although there is currently a wide variety of laboratory tests, a significant portion of pleural fluids of infectious origin remain undiagnosed,or diagnosis is exclusively based on clinical evidence because 30% to 35% of microbiologic studies on pleural fluids of Para pneumonic origin have a negative culture.[2]

In infections, fractions of the cell wall and other components associated with bacterial membrane can stimulate monocytes, macrophages, lymphocytes, and other cells present in the pleural space to liberate the different endogenous inflammatory mediators or cytokines responsible for the host response to these microorganisms.[3] Our objective was to find out if any of these pro-inflammatory cytokines, such as interleukin (IL)-6, IL-8, and IL-1β could help in the differential diagnosis of pleural fluids of nontuberculous infectious origin.

IL-6 is an immune system mediator that takes part in a large variety of biologic actions. It functions as a differentiation factor for B cells and acts as an activation factor of T cells.[4] Many types of cells, such as monocytes, macrophages, fibroblasts, endothelial cells, keratinocytes, T cells, and several tumor lines, can synthesize IL-6. Increased serum levels of IL-6 may be produced in different diseases, such as sepsis, autoimmune diseases, lymphomas, AIDS, liver cirrhosis, and in patients with infections or transplant rejection. [5]

IL-8 is a no glycosylated protein, and one of its most important biochemical functions is that it acts as a chemoattractant for neutrophils.[8] Initially, IL-8 was purified from monocytes, which are believed to be the major source of the protein, but many other cell types can synthesize IL-8, such as endothelial cells, epithelial cells, hepatocytes, fibroblasts, and chondrocytes. [6]

IL-1, an inflammatory cytokine mainly produced by activated macrophages and peripheral neutrophils, has an essential role in the activation of T cells. [7] It stimulates IL-2 production and secretion and the expression of IL-2 receptors by means of auxiliary cells. It stimulates the activation and differentiation of natural killer cells, fibroblasts, and thymocytes.

Material and Methods

This is a tertiary care hospital based cross sectional study which included 60 patients with pleural effusion. Patients were included in the study after getting informed consent from the patient and patient's attender. Also, an ethical committee approval was obtained. Clinical history was elicited and physical examination was performed. Pleural effusion was confirmed based on the chest X-ray finding. USG thorax/CT thorax was taken if the effusion was lobulated or minimal. After diagnosing pleural effusion, they were further divided into two categories, namely transudate and exudate based on Light's criteria. Pleural fluid was obtained by diagnostic pleural fluid aspiration (thoracentesis) after informed consent. Determination of biochemical parameters (LDH, glucose& protein) was performed usingsemi auto analyzer. Light's criteria for identifying exudate were as follows (at least 2 of the 3 to be fulfilled).

- Pleural fluid: Serum total protein ratio more than 0.5
- Pleural fluid: Serum LDH ratio more than 0.6

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- Pleural fluid LDH more than 200 IU/L

Diagnostic Tool

After differentiating exudates from transudates further diagnosis of exudative effusion – Tuberculous pleuritic were diagnosed if either the bacillus was isolated from the pleural fluid with predominant of lymphocytes and ADA>40. Malignant pleural effusion was diagnosed if malignant cells, either at cytology examination or in biopsy specimens were obtained. Para pneumonic pleural effusion and empyema were identified when there was an acute febrile illness with purulent sputum, pleuritic chest pain, pulmonary infiltrates and respond to antibiotic treatment or identification of the organism in the pleural effusion. Transudates - congestive cardiac failure, cirrhosis of liver and chronic kidney disease were diagnosed by further clinical features and investigations.

Pleural Fluid Interleukin 8 Level

The concentration of pleural fluid interleukin-8 was determined by ELISA (Enzyme Linked Immunosorbent Assay) method by using commercially available assay kits. To perform the test 50 micro liters of pleural fluid was needed. The pleural fluid samples were centrifuged immediately. The supernatant layers were frozen at −20°C. Manufacturer's recommendation and instruction were followed during performance of the test. The minimum cut off for detection of IL8 in the sample according to the manufacturer is 5pg/ml. Values greater than these were taken as significantly elevated as normally pleural fluid could not be obtained.

Statistical Analysis

All results are expressed as mean ± standard deviation (SD) for continuous variables and as frequencies for categorical variables. The difference in the age and gender between groups is disproved using independent student t-test and chi-square test, respectively. Mean pleural fluid Interleukin-8 level between the groups is analyzed using independent student t-test. The relationship between the variables of pleural fluid was estimated using the correlation graphs. An ROC curve was created in order to determine the specificity and sensitivity of interleukin-8. P-value< 0.05 is taken as being statistically significant. All analysis was done using Statistical Package for the Social Sciences (SPSS) version 26 for windows.

Result

Based on Light's criteria, of the 60 patients, 35 patients had exudative pleural effusion and 25 patients had transudate effusion. The demographic features of the included patients are shown in table 1. In the transudates subgroup, the maximum number of patients were between 41 to 60 years, whereas in exudates, the bulk of the study population consisted of the age group 21 to 40 years and the difference in the mean age between these two subgroups was found to be insignificant.

Table 1: Patient characteristics and etiology of pleural effusion (light's criteria based)

Patient characteristics (n=60)	Exudate group (n=35)	Transudate group (n=25)
Age distribution in years		
(21-40 years), n (%)	13 (38)	4 (17)
(41-60 years), n (%)	16 (45)	16 (61)
>60 years, n (%)	6 (17)	5 (22)
Total, n (%)	35 (100)	25 (100)

Table 2: Distribution of Sex

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Sex wise distribution	Exudate group (n=35)	Transudate group (n=25)
Male, n (%)	28 (80)	18 (72)
Female, n (%)	7 (20)	7 (28)

In both the subgroups, male patients contributed the bulk of the study population in table 2.

Table 3: Distribution of Etiology

Etiology, n (%)	Exudate group (n=35)	Transudate group (n=25)
Heart failure	10 (28)	14 (55)
Cirrhosis of liver	1 (4)	2 (8)
Chronic kidney disease	1 (4)	9 (37)
Abscess (lung, empyema, liver)	3 (7)	-
Para pneumonic effusion	1 (4)	-
Tuberculosis	14 (39)	-
Malignancy	3 (8)	-
Pancreatitis	1 (3)	-
Perforation peritonitis	1 (3)	-

Among the transudates subgroup, the maximum numbers (43%) were due to heart failure, followed by chronic kidney disease and cirrhosis of liver. 39% of exudates were due to tuberculosis followed by heart failure, malignancy, abscess, Para pneumonic effusion and abdominal causes in table 3.

Table 4: Comparison of Light's criteria biochemical parameters with Interleukin 8

Pleural fluid analysis	Transudate	Exudate	p value
Pleural Glucose (mgs %)	85.31 ± 24.34	57.64 ± 10.24	< 0.001*
Pleural LDH (IU/L)	220.24 ± 90.39	851.36 ± 346.71	<0.001*
Pleural Protein (gms %)	1.97 ± 0.40	4.18 ± 1.16	<0.001*
Pleural fluid protein/ Serum protein	0.25 ± 0.05	0.52 ± 0.11	<0.001*
Pleural fluid LDH/Serum LDH	0.29 ± 0.8	1.94 ± 0.92	<0.001*
IL-8 (pg/ml)	302.21 ± 48.16	3109.34±314.67	<0.001*

The parameters in Light's criteria (pleural fluid protein and LDH) and Interleukin 8 were able to differentiate transudate from exudate and were found to be statistically significant with p value of < 0.001. Interleukin-8 had a significant positive correlation with LDH ($r = 0.53$, $p < 0.001$) and protein ($r = 0.49$, $p < 0.001$) in pleural fluid. Analysis of variance (ANOVA) was done to find out whether there was any difference in IL-8 concentrations seen among various diseases in patients with exudates in table 4.

Table 5: Mean values of IL-8 in various exudative pleural effusion

Diseases	n	IL-8 Mean ± SD
Systemic Failure effusion (heart failure, Liver cirrhosis, CKD)	12	1288.67 ± 1520.34
Abscess (Empyema, Liver abscess, Lung abscess)	4	9321.64 ± 301.24
Malignancy	4	3298 ± 473.61
Surgical effusions (Pancreatitis, peritoneal perforation)	2	3198 ± 1312.29
Para pneumonic effusions	2	1768 ± 349.29
Tuberculosis	11	3328.27 ± 1691.29

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Our study results showed that there was significant difference seen in mean IL-8 concentrations in the abscess subgroup (empyema, liver and lung abscess) compared to rest of the diseases in exudates group in table 5.

Discussion

Pleural effusion is most frequently seen during the course of pneumonias. Pleural effusion can be seen due to the intrusion of an infectious agent or an irritating foreign substance inside the pleural cavity or due a direct access of harmful materials or neoplastic cells into the pleural cavity via hematogenous route.[8] It can be observed following pleural trauma or in association with asbestosis related pleural diseases. Pleural effusion or empyema in association with pneumonia is relatively frequent.[9] Pleural effusions are known to develop secondary to trauma, cardiac, renal, collagenous diseases and malignancies besides pneumonia.[10]

From 61% to 80% of the cases with pleural effusions are of infectious origin. De-spite the usage of broad spectrum antibiotics, empyema continues to be an important health care problem during childhood. Empyema is also an important cause of mortality in the developing countries. In the remaining 20%– 39% of the cases, empyema develops secondary to trauma, malignancies, and renal diseases.[11] In our study group an infectious agent was responsible for 75% of the cases. Many studies have been conducted concerning the levels of IL-1β, IL-2, IL-6, and IL-8 in body fluids in various infections. Although many studies investigating the alterations in levels of IL according to the exudative or transudate nature of pleural effusions in adults have been performed, similar studies pertaining to children are relatively few.[12-22]

IL-8 is the mediator and the regulator of chemo-taxis of leukocytes in inflammatory processes. In adults, miscellaneous studies have analyzed IL-8 levels in pleural fluids. IL-8 levels were found to be higher in cases with infectious pleural effusions compared with the patients with noninfectious effusions.[23] Ceyhan et al [24] reported higher levels in emphysematous/Para pneumonic effusion groups rather than the tuberculous group. However, Antony et al [25] detected higher levels in cases with Para pneumonic effusions unlike those found in the group with tuberculous effusions. IL-8 concentrations in the emphysematous group were reported to be higher than those measured in the Para pneumonic group. In accordance with this study, in our study the highest levels of IL-8 were detected in the emphysematous, Para pneumonic, and tuberculous pleural effusions in decreasing order. Dlugovitzky et al [26] discovered statistically significantly higher IL-8 values in the tuberculous pleural effusion group in contrast with findings in the Para pneumonic pleural effusion group. Ashitani et al [27] and Broaddus et al [28] found higher levels in the empyema group compared with the other groups. Miller and Idell [29] have detected significantly higher levels in the exudate group rather than the transudate group. In this study, similar to the findings of other investigations, IL-8 levels in the exudate group were reported to be 28 times higher than those found in the transudate group.

This study have shown that for the differentiation between the exudative and transudate pleural effusions, in addition to the parameters such as protein, glucose and LDH, pleural fluid IL-1β, IL-2, IL-6, and IL-8 levels could be used. Etiological factors can be differentiated by deter-mining pleural fluid IL levels. Taking these levels into consideration, the exudative or transudate nature of pleural fluids can be ascertained. Accordingly, a definitive diagnosis, a successful treatment and reduction in mortality can be achieved

Conclusion

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Pleural fluid IL-8 is a marker of differentiation with a diagnostic accuracy at least similar to that of classical parameters such as pH, glucose of LDH levels.Although the classical parameters have a better specificity and sensitivity than IL-8, its value is important because it is only one parameter for determination versus three biochemical parameters (LDH, glucose and pH) and because it has a specific method for taking pH and its determination is only by the blood gas machine. IL-8 may be used as an alternative marker for the complication of Para pneumonic effusion but its diagnostic value needs further analysis.

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