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BIOCHEMICAL ANALYSIS OF PLEURAL FLUID IN MALIGNANT PLEURAL EFFUSIONS: A PROSPECTIVE STUDY IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: Malignant pleural effusion (MPE) is a common and debilitating clinical manifestation in the patients with metastatic and advanced stage of neoplastic disease. This prospective study was designed in an endeavor to scrutinize various biochemical parameters like pH, glucose, protein, albumin and adenosine deaminase (ADA) in an array of malignant pleural effusions. **Methods:** Patients with malignant pleural effusions were diagnosed by thoracentesis through cytology and pleural needle biopsy. **Results:** The low pH levels in pleural fluid were associated with significantly lower pleural fluid glucose levels in malignant pleural effusions. The pleural fluid protein levels were ≥ 3.1 g/dL and the albumin levels ranged from 1.1 to 3.9 g/dL in malignant (exudative) pleural effusions. Furthermore, the pleural fluid ADA values (≤ 40 U/L) seldom exceeded the diagnostic cut-off for tuberculosis in either primary/metastatic malignant pleural effusions. Combination of these biochemical parameters considered in the present study can be used to deduce malignant pleural effusions, even if they are cytologically negative and should be subjected for repeated cytology or advanced work up for malignant primary. **Conclusion:** We could adjudicate that this study adds value to the existing knowledge of the pulmonologists about the biochemical profile of malignant pleural effusions, thereby ensuing in a more appropriate treatment intervention according to the histological variants of malignancy.

Keywords: Malignant pleural effusion, thoracentesis, cytology, needle biopsy.

INTRODUCTION

Pleural effusion is an anomalous buildup of fluid in the pleural space which is commonly encountered by chest physicians. Pleural effusion often presents as a common diagnostic problem as no cause may be found in about 20% of cases, in spite of careful evaluation. The prominent etiologies of pleural effusion are infections (eg: pneumonia, tuberculosis) and malignancy. But, the clinical, biochemical and cytological parameters of plural effusions pose a significant diagnostic dilemma due to the shared features between the tubercular and malignant effusions, both being exudates and chiefly lymphocytic effusions.

In a meticulous review done by Muduly *et al*., [1], it has been reported that various neoplasms of lung, ovary, breast, and lymphomas comprise more than 75% of cases of malignant pleural effusion (MPE). Malignant pleural effusions which encompass a heterogeneous group of conditions embody an important source of morbidity in the patients with underlying cancer. They are frequently the primary manifestation and diagnostic source of malignancy in patients with previously diagnosed malignancies, or

cancer recurrence after therapy, and also provide prognostic information.

Diagnosis of malignant pleural effusions is generally established by demonstrating malignant cells in pleural fluid or in pleura itself. But, the diagnostic yield is low and variable with both these procedures, 40-70% with cytology, 39-75% with needle biopsy of parietal pleura [2]. Moreover, malignant lymphocytes in pleural fluid could not be distinguished from chronic inflammatory cells thus limiting the diagnostic utility of cytology.

Numerous diagnostic procedures have been offered, such as immuno histochemical staining with monoclonal antibodies to tumor markers including flow cytometry, chromosomal analysis of malignant cells, pleural fluid hyaluronic acid levels, LDH isoenzymes assay, and tumor marker assays, to differentiate between benign and malignant effusions [3].

Despite the availability of all tests, it might be necessary to exploit more invasive diagnostic tools like pleural biopsy or thoracoscopy to establish a diagnosis. Hence, there is still a need for defining the most cost effective approach for the quick and early diagnosis of exudative pleural effusions in malignancy. This prospective study was designed in an endeavor to analyze the profile of various biochemical parameters like pH, glucose, albumin and adenosine deaminase (ADA) in an array of malignant pleural effusions, by using diagnostic thoracentesis with cytology and pleural needle biopsy.

MATERIALS AND METHODS

Study design and population

This institution based prospective study was conducted on 54 patients with suspected malignant pleural effusions attending or admitted to the Department of Pulmonary Medicine, Mediciti hospitals, Hyderabad between September 2008 and August 2010. Only those patients with a confirmed pleural malignancy and biochemical analysis of pleural fluid determined at initial thoracentesis were included in the study group. Exclusion criteria were Frank empyema and patients not willing to participate in the study.

Data collection

All the subjects were interviewed with detail clinical history and underwent complete general and systemic examinations, according to our pre-designed and pre-tested proforma. Informed consent was obtained from the patients for participated in this study.

METHODS

Besides the routine investigations, the patients were subjected to the chest radiography (PA view), diagnostic thoracentesis [4] and pleural needle biopsy.

After thoracentesis, all the patients were inspected for any evidence of pneumothorax. If the clinical suspicion was high, chest X-ray was done and necessary intervention was made. Needle biopsy of pleura using Abraham's pleural biopsy needle was performed once when malignant effusions were suspected.

Pleural fluid was collected for the pH determination in a manner analogous to that for arterial blood gases. All the samples were collected anaerobically in a syringe rinsed with 0.2 mL of heparin (1:1000) and kept on ice until analyzed; pH was determined within 30 minutes of thoracentesis.

The collected pleural fluid specimens were immediately carried to the biochemistry and pathology laboratories. Positive diagnosis of malignancy was based on either cytology or biopsy for malignant cells.

Effusion pH and also protein, albumin, glucose, and LDH present in the pleural effusions were measured by multichannel analyzer (Siemen's DADE Behring Dimension Xpand Plus) after thorough calibration of the analyzer.

The fluid for ADA quantification was centrifuged at 3000 revolutions per minute for

20 minutes at 4°C. The supernatant was stored at -70°C until analysis and measured by the calorimetric method of Guisti [5].

RESULTS

Age and Body Mass Index (BMI)

In our study of 54 patients (33 males and 21 females), majority of the patients were above the age of 40 years and the mean age of all the patients in the present study was about 56 ± 12 years. Also, 50 (92.59 %) cases of the total patients had a normal BMI.

Histological spectrum of malignancies

In our study , 77.77% of the total patients presented primary lung carcinoma, of them majority of the cases were of adenocarcinoma constituting around 37.04 % followed by 20.37 % of squamous cell carcinoma , 16.67 % of malignant mesothelioma and only 2 cases (3.70%) of large cell carcinoma [Table 1]. Besides, 22.23 % of the total patients presented metastatic malignancies, of which 11.11 % were of breast carcinoma and 5.56 % each of ovarian carcinoma and unknown primary carcinoma [Table 1].

pH in malignant pleural effusions

In our study, among lung malignancies, the value of pleural fluid pH ranged from 6.6 – 8.5. In 70 % cases of adenocarcinoma, 63.64 % cases of squamous cell carcinoma, 100 % cases of large cell carcinoma and 89 % of cases of malignant mesothelioma, pH was acidic [pH ≤ 7.3]. Among metastatic malignancies the value of pH ranged from 7 – 8. In 50 % cases of breast carcinoma and in only 33.33 % of cases of ovarian carcinoma pH was acidic. Overall, 64.81 % of the total patients had a pH of < 7.4, among them 27.78 % had a pH < 7. 35.19 % of the total patients had a pH > 7.4, among them 2 patients had a pH of > 8.1 [Table 2].

Glucose in malignant pleural effusions

It was observed in our study that, among lung malignancies, the pleural fluid glucose ranged from 12 to 116 mg/dL. In 45 % cases of adenocarcinoma, 63.64 % cases of squamous cell carcinoma, 100 % cases of large cell carcinoma and 89 % cases of malignant mesothelioma, pleural fluid glucose was ≤ 60 mg/dL. Even among metastatic malignancies the pleural fluid glucose similarly ranged from 26 to 110 mg/dL. In 50 % cases of breast carcinoma and 33.33 % cases of ovarian carcinoma, pleural fluid glucose was ≤ 60 mg/dL. Overall, 53.70 % of the total patients had a pleural fluid glucose ≤ 60 mg/dL and 46.30 % of the individuals had glucose of ≥61 mg/dL [Table 3].

pH and glucose relationship in malignant pleural effusions

In our study, in almost all cases of lung malignancies and metastatic malignancies with a low pH (≤ 7.3), the glucose value was also noted to be low (≤ 60 mg/dL). Except among 35.71% cases of adenocarcinoma with a low pH, the glucose value was noted to be above 60 mg/dL and the rest of all other lung and metastatic malignancies had a normal and above normal pH with normal glucose [Table 4].

Protein in malignant pleural effusions

Among lung malignancies, the pleural fluid protein ranged from 4 to 7.1 g/dL. Among metastatic malignancies, the pleural fluid protein ranged from 4.4 to 7 g/dL. In general, all the lung and metastatic malignancies in the present study had a pleural fluid protein of ≥ 3.1 g/dL, i.e all the cases in the present study met the Light's criteria based on absolute value of pleural fluid protein to be classified as exudative pleural effusions and did not require the additional consideration of LDH to be categorized as exudative effusions [Table 5].

Albumin in malignant pleural effusions

In this study, among lung malignancies, the pleural fluid albumin ranged from 1.1 to 3.9 g/dL , with the extremely low levels not confined to a particular lung malignancy. i.e., almost all the lung malignancies equally have very low albumin levels. Among metastatic malignancies the pleural fluid albumin ranged

from 1.6 to 3 g/dL, with the low levels not confined to a particular metastatic malignancy. Overall 74.07 % of the total patients had a pleural fluid albumin value ≤ 2.5 g/dL, of which 48.15 % of them were males and 25.93 % of them were females [Table 6].

ADA in malignant pleural effusions

In our study, among lung malignancies the pleural fluid ADA ranged from 2 to 68 U/L. ADA ≥ 40 U/L was noted in 3 cases of lung malignancies of which 2 were adenocarcinoma; with ADA of 48 U/L in one and 46.4 U/L in the other and in 1 case of malignant mesothelioma with ADA of 68 U/L. Among metastatic malignancies, the pleural fluid ADA ranged from 15 to 52 U/L. ADA ≥ 40 U/L was noted in 2 cases of metastatic malignancies of which 1 was breast carcinoma with ADA of 52 U/L and other case of ovarian carcinoma with ADA of 50 U/L. Overall among the total 54 patients, 90.74 % of the cases had pleural fluid ADA level ≤ 40 U/L, only 5 of 54 cases had a ADA value ≥ 40 U/L of which 2 were adenocarcinoma, 1 malignant mesothelioma, 1 breast carcinoma and 1 ovarian carcinoma [Table 7].

DISCUSSION

Pleural effusion is frequent sequelae in the patients with metastatic and advanced stage of neoplastic disease. In majority studies, lung carcinoma has been the most common malignancy, accounting for approximately one third of all malignant effusions. In our study, the mean age was 56 ± 12 years which was compared to the age group of patients in other studies [6-8]. The slightly lower age group in our study can be because of the higher no. of malignant mesothelioma cases which present at a relatively younger age.

The malignancy is one of the common causes of exudative pleural effusions. The results of several reviews indicated that in men, lung carcinoma is the most common metastatic tumor to the pleura. Lung and breast cancer together, accounts approximately 50–65% of all malignant effusions [9]. Tumors of lymphocytes, gastrointestinal and genitourinary tract, collectively accounts for further 25%. Pleural effusions from an unknown primary are responsible for 7–15% of all malignant pleural effusions [10]. In comparison to the above mentioned reports, our study included 54 patients with malignant pleural effusions .The primary lung malignancy constituted about 77.78% of the total patients, of which 37.04% were of adenocarcinoma, 20.37% of squamous cell carcinoma, 16.67 % of malignant mesothelioma and only 2 cases of large cell carcinoma. 22.22% of the total patients presented metastatic malignancies, of which 11.11% were of breast carcinoma and 5.56% each of ovarian carcinoma and unknown primary.

In a non-concurrent cohort study carried out by Eva *et al* [11] in which patients with malignant pleural effusion were evaluated which included 38.3% non-small-cell lung cancer, 26.6% breast cancer , adenocarcinoma, unknown primary site in 12.5%, non-Hodgkin's lymphoma in 8.3% and 1.6% of malignant pleural mesothelioma.

Our data from this study is in harmony with that of Hsu *et al* [12], Sears *et al* [13], and Johnston *et al* [8], who demonstrated lung malignancy in males and breast malignancy in females are the most important causes of malignant pleural effusions. 5.56% of our cases were not able to determine the primary which was appeared to be considerably lower than that of Sears *et al* [13] (12.8%), Johnston *et al* [8] (10.16%), Salyer *et al* [14] (17.8%).

Observations from our study displayed that the value of pleural fluid pH of lung and metastatic malignancies range from 6.6 – 8.5. 64.81% of our study patients had an acidic pH, with a mean pH of 7.21 ± .02 which was comparable to the pH demonstrated in other studies [6, 9]. A malignant pleural effusion with low pleural fluid pH results when the pleural space is extensively

involved with tumor and fibrosis. Measurement of pleural fluid pH in a malignant pleural effusion provide the clinician with information that can be used to proffer a plan for further diagnostic testing, prognostic information for the patient, and a rational approach to palliative therapy.

53.70% of our total patients with malignant pleural effusions depicted a low glucose which was comparable to numerous studies (61.7%) [15,16]. Patients with malignant pleural effusions and a low glucose level portray that they have a greater tumor burden in their pleural space than do those with normal pleural fluid glucose levels.

With respect to pH and glucose correlation, it suggests that the physico-chemical processes leading to this phenomenon are interrelated. Studies have shown that the metabolic activity of low-pH malignant pleural fluid is similar to normal-pH malignant pleural fluid, implying that the pleural fluid *per se* is not responsible for the biochemical changes [17]. There is impaired glucose transfer from blood to pleural fluid and from pleural fluid to blood in patients with low-pH but not normal-pH malignant effusions. Due to the anomalous pleura in patients with low-pH effusions, the rate of transport of CO_2 and lactic acid out of the pleural space is slowed, and accumulation occurs, resulting in a diminished pleural fluid pH. Similarly, even in our study, 35.71% of cases of adenocarcinoma, had a low pH with relatively normal glucose values which probably could be attributed to a laboratory error.

Clinical studies conducted by Ryu *et al* [10] and Porcel *et al* [18] reveal that most MPEs by the criteria of protein and LDH were exudates according to Light criteria; 3%-10% were transudates, where as in comparison 100% of our effusions were exudative. The possible explanation, the lymphatic system of the parietal pleura plays a pivotal role in the resorption of pleural liquid and protein. Interference with the integrity of the lymphatic system between the parietal pleura and mediastinal lymph nodes in malignancy can culminate in a pleural effusion with elevated protein levels. Our data shows that, 74.07% of the total patients having a pleural fluid albumin value ≤ 2.5 g/dL, ranging from 1.1 to 3.9 g/dL in the present study was comparable to the study conducted by Dhar *et al*, [15] in which the pleural fluid albumin ranged from 0.5 to 3.9 g/dL.

In line with other studies, our data displayed that 90.74% of the total patients in the present study demonstrate ADA levels < 40 [19, 20]. The plausible reason underpinning the higher ADA levels in some lymphocytic effusions than others would be that the quantity of ADA is related to the amount of T - lymphocytes present, the ADA activity varies dependent on different pathologic conditions, such that the ADA activity is highest with TB, and also lymphoma. However, our study did not consider the lymphocytes or monocyte counts so it fails to demonstrate any correlation between monocytes, total lymphocytes, or T-cell subtypes with either total ADA or its isoenzyme levels.

We admit the following limitations from our study: limited samples were included in our study with only certain malignancies under consideration. This study was based on established basic parameters and did not include any novel or molecular parameter in viewing the biochemical profile of malignant pleural effusions. As the study being a point observational study of profile of biochemical parameters in malignant pleural effusions, the treatment details for these malignant pleural effusions, severity of the disease, tumor burden and prognosis have not been ruled out.

CONCLUSIONS

The pH of pleural fluid in malignant pleural effusions was found to be acidic (pH<7.4). The low pH levels in pleural fluid were associated with significantly lower pleural fluid glucose

levels in malignant pleural effusions. The pleural fluid protein levels were relatively higher in malignant (exudative) pleural effusions. Furthermore, the pleural fluid ADA values rarely exceeded the diagnostic cutoff for TB (more than (>40)) in either primary/metastatic malignant pleural effusions.

Despite the limitations of our study, combination of the above biochemical parameters considered in the present study can be used to deduce malignant pleural effusions even if they are cytologically negative and should be subjected for repeated cytology or further work up for malignant primary. We strongly recommend that a larger study should be conducted with regular follow-ups with supplementary data on the details of therapeutic interventions.

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Table -1: Histological spectrum of malignancies

Malignancy Type		No. of patients (%)
Lung	Adenocarcinoma (AC)	20 (37.03)
	Squamous cell carcinoma (SCC)	11 (20.37)
	Large cell carcinoma (LCC)	2 (3.70)
	Malignant mesothelioma (MM)	9 (16.67)
Metastatic	Breast	6 (11.11)
	Ovary	3 (5.56)
	Unknown Primary (UP)	3 (5.56)

Table – 2: pH in malignant pleural effusions

Malignancy Type	No. of patients (%)	pH Values		
		Lowest	Highest	Mean ± SD
AC	20 (37.03)	6.6	8.5	7.26 ± 0.38
SCC	11 (20.37)	6.9	8.5	7.36 ± 0.49
LCC	2 (3.70)	6.9	7.2	7.05 ± 0.21
MM	9 (16.67)	6.8	7.4	7.07 ± 0.21
Breast	6 (11.11)	7.0	8.0	7.38 ± 0.38
Ovary	3 (5.56)	7.2	7.6	7.40 ± 0.20
UP	3 (5.56)	7.4	8	7.66 ± 0.31

Table – 3: Glucose in malignant pleural effusions

Malignancy Type	No. of patients (%)	Glucose Values (mg/dL)		
		Lowest	Highest	Mean ± SD
AC	20 (37.03)	12	105	62.3 ± 26.81
SCC	11 (20.37)	30	116	59.36 ± 32.16
LCC	2 (3.70)	23	55	39 ± 22.63
MM	9 (16.67)	15	66	31.89 ± 16.51
Breast	6 (11.11)	26	110	70.17 ± 38.89
Ovary	3 (5.56)	66	110	85.3 ± 22.48
UP	3 (5.56)	86	90	88.67 ± 2.31

Table – 4: pH and glucose relationship in malignant pleural effusions

Range	AC	SCC	LCC	MM	Breast	Ovary	UP
pH <7.4 & Glucose ≤60	9	7	2	8	3	-	-
pH <7.4 & Glucose >60	5	-	-	-	-	1	-
pH = 7.4 & Glucose > 60	6	4	-	1	3	2	3

Table – 5: Protein in malignant pleural effusions

Malignancy Type	No. of patients (%)	Protein values (g/dL)		
		Lowest	Highest	Mean ± SD
AD	20 (37.03)	4.0	7.1	5.22 ± 0.89
SQC	11 (20.37)	4.0	6.4	5.14 ± 0.82
LCC	2 (3.70)	4.5	5.0	4.75 ± 0.35
MM	9 (16.67)	4.5	6.6	5.68 ± 0.69
Breast	6 (11.11)	4.4	7.0	5.9 ± 0.85
Ovary	3 (5.56)	5.1	6.0	5.5 ± 0.46
UP	3 (5.56)	5.0	5.6	5.33 ± 0.31

Table – 6: Albumin in malignant pleural effusions

Malignancy Type	No. of patients (%)	Albumin Values (g/dL)		
		Lowest	Highest	Mean ± SD
AD	20 (37.03)	1.1	3.0	2.18 ± 0.56
SQC	11 (20.37)	1.4	3.2	2.13 ± 0.65
LCC	2 (3.70)	2.2	2.0	2.5 ± 0.42
MM	9 (16.67)	1.1	3.9	2.03 ± 0.85
Breast	6 (11.11)	1.6	2.8	2.1 ± 0.43
Ovary	3 (5.56)	2.0	3.0	2.47 ± 0.50
UP	3 (5.56)	2.0	2.6	2.2 ± 0.35

Table – 7: ADA in malignant pleural effusions

Malignancy Type	No. of patients (%)	ADA Values (U/L)		
		Lowest	Highest	Mean ± SD
AD	20 (37.03)	2	48	25.5 ± 11.70
SQC	11 (20.37)	10	35	22.36 ± 8.71
LCC	2 (3.70)	5	10	7.5 ± 3.54
MM	9 (16.67)	9	68	32.45 ± 16.49
Breast	6 (11.11)	16	52	29.5 ± 12.68
Ovary	3 (5.56)	18	50	33.33 ± 16.04
UP	3 (5.56)	15	30	23.67 ± 7.77