

Diagnostic utility of neuron specific enolase (NSE) in serum and pleural fluids from patients with lung cancer and tuberculosis

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Abstract: Several past and recent investigations have focused on the determination of tumor markers in pleural fluids to assess their usefulness as less invasive replacement method of diagnosis. In this regard, few studies have dealt with the determination of the tumor marker, neuron specific enolase (NSE), in pleural fluids of patients suffering from both benign and malignant diseases such as non small cell lung carcinoma (NSCLC), small cell lung carcinoma (SCLC) and tuberculosis. Therefore, the present study was undertaken to establish the diagnostic utility of NSE in malignant condition by assessing levels in serum and pleural fluids of patients with lung cancer and by comparing it with a benign pulmonary disease of tuberculosis. Pleural fluids were obtained from 22 patients with carcinomatous pleurisy due to SCLC, 11 patients with carcinomatous pleurisy due to non-small cell lung cancer, and 30 patients with tuberculosis pleurisy for comparison purpose. Determination of NSE levels was performed by ECL technology according to the manufacturer's instructions. NSE levels of pleural fluids from SCLC patients were significantly elevated ($P < 0.0001$) when compared with pleural fluids from NSCLC and tuberculosis patients. Moreover, pleural fluids of all 30 tuberculosis patients and 11 NSCLC patients showed moderate significance ($P < 0.05$ and $P < 0.01$, respectively) when compared with each other. In addition, cumulative results of NSE levels from SCLC and NSCLC combined also showed high significance ($P < 0.001$) as compared to pleural fluids of tuberculosis patients and moderate significance ($P < 0.01$) when compared with serum levels of both malignant and benign groups. It is concluded that determination of NSE levels in pleural fluids of lung cancer patients noted to be an effective diagnostic tool to differentiate carcinomatous pleurisy due to SCLC from those occurring due to NSCLC and tuberculosis. Further studies with larger group of patients are under progress to further establish and strengthen its diagnostic specificity and sensitivity.

Keywords: Neuron specific enolase (NSE), small cell lung carcinoma (SCLC), non-small cell lung carcinoma (NSCLC), pleural effusion.

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INTRODUCTION

In a diagnostic setting, differentiation of malignant and non-malignant pleural effusions or fluids is of great importance; however, currently associated techniques are either insufficient or invasive¹⁻³. Moreover, it is documented that a malignant pleural effusion may be the initial presentation of cancer in 10% to 50% of patients^{4,5}. It was also reported that a considerable quantity (approximately 20%) of pleural effusions is due to a particular malignant condition; 50% of which is reported to be due to primary lung cancer^{4,6}. Several investigations during last two decades have focused on the detection and use of reliable tumor markers, such as CEA, NSE, CYFRA 21-1 and TPS in pleural fluids, as a less invasive replacement method of diagnosis^{3,4,7-10}.

In this regard, neuron-specific enolase (NSE) which is the neuronal form of the glycolytic enzyme enolase found in extracts of brain tissue, neuroendocrine cells and neuroendocrine tumors including SCLC, has been investigated lately^{9,11,12,14,15}. Some studies have dealt with NSE levels of pleural fluid in diseases such as NSCLC, SCLC and benign pulmonary disease such as tuberculosis^{4,3,9}. NSE is well regard as a serum

marker of small cell lung carcinoma and SCLC pleural effusions and fluids^{4,15,16}. NSE levels were also found to be correlated with the histological confirmation of SCLC and comparatively exhibited high sensitivity in SCLC as compared to other markers^{12,14}. However, it was argued that the sensitivities of pleural fluid NSE for the diagnosis of malignant effusion from carcinomas including SCLC were relatively low^{4,17}. Therefore, in the present study, we have evaluated NSE levels in serum and pleural fluids of patients with pulmonary cancer and compared the data with NSE levels of tuberculosis pleurisy to determine its diagnostic utility and efficacy.

MATERIALS AND METHODS

Research design

In this prospective study, 63 consecutive patients with pleural fluids both with malignant and benign conditions were included. Their samples, both serum and pleural fluid, were referred to Department of Pathology, Govt Layri General Hospital, Karachi and Department of Biochemistry Lab services at Liaquat National Hospital and Medical College, Karachi, during January 2006 to December 2009. The underlying diseases reported,

clinical signs and symptoms, physical examination, chest X-ray, CT-scan, biochemical, cytological and bacteriological analysis of pleural fluid, were obtained through records.

Samples and NSE analysis

Eight ml venous blood and 8-10 ml pleural fluids were collected from each patient. The samples were centrifuged immediately at 2000xg, and then the serum and pleural fluid were kept at -20°C until further use. Determination of NSE levels was performed by ECL technology (Elecys 2010, Roche Diagnostic, Basil) according to the manufacturer’s instructions. Previously established protocols were followed for processing of various steps^{3,4,9}. The cut-off levels NSE for differentiation of benign and malignant pleural effusions/fluids were 5.21 ng/ml and 13.00 ng/ml, in pleural fluids and sera, respectively⁹. Pleural fluids was obtained from 22 patients with carcinomatous pleurisy due to SCLC, 11 patients with carcinomatous pleurisy due to non-small cell lung cancer, and 30 patients with tuberculosis pleurisy, confirmed by pleural biopsy or bacteriologic or cytologic study of effusions.

Statistical analysis

All data are expressed as means and standard deviations (SD). Statistical analysis was performed using statistical software (SPSS ver 13 Chicago, IL). Differences between the two groups were evaluated using the student “t” test and Pearson’s correlation.

RESULTS

Among the 63 individuals, 30 fluids were defined as benign effusions of tuberculosis pleurisy, and 33 were defined as malignant fluids associated with primary pulmonary cancer of SCLC (n=22) or NSCLC (n=11) origin. Out of the 30 patients with benign effusions, 21 were male (mean age, 59 years) and 9 females (mean age 61 years). Out of 33 patients with malignant effusion, 25 were male (mean age, 59 years) and 8 were females (mean age 63 years).

Table 1: Cumulative NSE levels in patients with benign and malignant effusions.

Variables	Malignant Effusion (SCLC & NSCLC) (n=33)	Benign Effusion (Tuberculosis) (n=30)
Serum	53.10±18.10	12.66±4.82*
Pleural effusion/fluid	59.21±20.42	9.12±2.82**

*p<0.001, **p<0.0001

Table 1 represents the cumulative NSE levels in patients with malignant and benign effusions, whereas table 2 detailed the etiology and percentage distribution of pleural effusions with respect to

disease conditions. Mean and SD of NSE in pleural fluids from 22 patients with cytology-positive SCLC, 11 patients with NSCLC and 30 patients with tuberculosis pleurisy are shown in Table 3.

Table 2: Etiology of pleural effusions in patients with malignant and benign conditions.

Cause	Number (n)	Percentage (%)
Malignant		
SCLC	22	34.92
NSCLC	11	17.46
Benign		
Tuberculosis effusion	30	47.61
Total	63	100.00

Table 3: Mean and SD of NSE (ng/ml) in Pleural Fluids from SCLC, NSCLC, and Tuberculosis

Patient Group	Mean±SD
Cytology-positive SCLC (n=22)	79.21 ± 20.26*
NSCLC (n=11)	17.00 ± 9.22**
Tuberculosis (n=30)	9.00 ± 1.60***

*p<0.0001, **p<0.01, ***p<0.05

Table 4: NSE (ng/ml) in serum from SCLC, NSCLC and tuberculosis

Patient Group	Mean±SD
Cytology-positive SCLC (n=22)	86.40±22.12*
NSCLC (n=11)	24.22±6.20**
Tuberculosis (n=30)	17.62±11.21**

*p<0.001, **p<0.05, ***p<0.05

NSE values in SCLC patients were significantly elevated (P<0.0001) when compared with those of NSCLC and tuberculosis. Mean±SD value of NSE in tuberculosis pleural effusion was 9.12 ng/ml and in serum 12.66 ng/ml. Cumulative value of NSE from both SCLC and NSCLC patients were 53.10 ± 18.10 ng/ml in serum and 59.21 ± 20.42 ng/ml in pleural effusions. Pleural effusion of all 33 tuberculosis patients and 11 NSCLC patients showed moderate significance (P < 0.05 and P < 0.01, respectively) when compared with each other. However NSE levels in SCLC patients showed 89.1 % positivity and a significance level of P < 0.0001 when compared with NSE levels of benign disease. In serum, NSE levels were found to be slightly higher but comparable with respect to their non-significant difference with the results obtained in pleural fluids (Table 4).

DISCUSSION

In present study we evaluated the diagnostic utility of NSE in lung cancer and tuberculosis pleurisy to differentiate malignant from benign effusions. NSE level of pleural effusion in SCLC patients’ was significantly higher than in NSCLC and most definitely than tuberculosis pleurisy. The

determination of tumor markers, such as NSE in serum and pleural fluid has been proposed as an alternative way of establishing the diagnosis of malignant pleural effusion and it is a known marker of SCLC^{3,4,8,18,19}.

The usefulness of its determination in pleural fluid to distinguish SCLC from NSCLC has been demonstrated earlier^{4,15}. In present study, which is conducted with a group of diagnosed cases of lung cancer patients, elevated levels of NSE in pleural fluid were found in all cytology-positive SCLC patients. The results are in accordance with previously reported studies which showed elevated NSE levels in serum from SCLC patients (65%-94%) and pleural effusion^{9,11}.

It has been reported that only 8.5 to 29.0% of patients with NSCLC showed raised serum NSE levels^{20,21} and thus possess definite specificity for the pleural fluid NSE levels in SCLC when compared to serum NSE levels. Two studies are also suggestive of the same fact that NSE had the highest sensitivity in SCLC and higher levels always corresponds to the confirmation of SCLC as compared to NSCLC^{12,15}.

It has been reported that efficacy of NSE levels in pleural effusion in other clinical situation may be of some use in the staging of a patient with SCLC, who had a pleural effusion with negative cytological findings^{9,19}. Previously, it was reported that NSE was significantly elevated in malignant pleural fluids of certain patients with pulmonary malignancy^{9,22}.

In present study we have also observed significant difference in NSE levels in malignant and benign conditions; however, it is not the case in all studies conducted previously in which the researchers did not observe any difference between NSE level in malignant and benign conditions²⁴. Furthermore, in agreement with our study, a much higher sensitivity and specificity of NSE has been reported in pleural fluid despite a raised cut-off levels²⁴.

Therefore, besides cut-off level, other factors might interfere with the observed specificities and sensitivities. Previous studies on usefulness of NSE in pleural effusion as a diagnostic tool reported a wide range of cut-off values ranging from 8.8 ng/ml to a highest of 26.00 ng/ml^{4,15,22}. The cut off value of 26.00 ng/ml was argued to be high as compared to healthy control subjects due to destruction of blood cells in exudates pleural effusions.

Other tumor marker such as cancer antigen 15-3 (CA 15-3), CYFRA 21-1, TPS and CEA have also been examined for their diagnostic utility in malignant and benign pleural effusions^{10,12,13}.

In this regard, the mean serum and pleural fluid levels of CYFRA 21-1 in squamous cell carcinoma were slightly higher than those in adenocarcinoma and small cell carcinoma⁴.

Several researchers have also evaluated the value of various combinations of several markers. For example, it was noted that the determination of NSE in pleural fluid improved the sensitivity of CEA alone, which is consistent with a previous findings²⁵. In pleural fluid, the determinations of both CEA and NSE were rather superior to those of CEA, NSE, and CYFRA 21-1 individually.

In serum, concurrent measurements of CYFRA 21-1 and CEA increased both sensitivity and specificity versus CEA alone, whereas the addition of NSE to CEA plus CYFRA 21-1 increased sensitivity but not specificity. However, diagnostic accuracy of measuring all three markers in serum was slightly better than any other combination. In a previous study, it was suggested that TPS and CYFRA 21-1 are useful serum marker for the diagnosis of NSCLC, whereas NSE assessment is significant for monitoring course of patients' prognosis and management in SCLC¹⁰.

A slightly raise NSE concentration was also noted in pleural exudates of several lung cancer patients as compare to those obtained from non-tumorous pleurisy, whereas CYFRA 21-1 was noted to be significantly raised in pleural effusions than in serum samples. Furthermore, in another study, the scientists observed the usefulness of CA 15-3 in diagnosis of malignant pleural effusions where it showed its efficacy in differentiating lung cancer from other etiologies of pleural effusion^{3,8}.

It was also suggested that the best combination of tumor markers which revealed 100% specificity and 100% PPV with 76.5% sensitivity could be obtained by measurement of CA 15-3 in serum and pleural fluid and later adding assessment of NSE in pleural fluid³. A higher sensitivity (80%) with the same level of specificity (100%) could be obtained by additional measurement of CEA in pleural fluid³.

CONCLUSION

The determination of NSE levels in pleural fluid seems to be an effective diagnostic tool to differentiate carcinomatous pleurisy due to SCLC from those due to NSCLC and tuberculosis. A further study with larger group of patients is under progress to strengthen diagnostic specificity and sensitivity of NSE in same setting.

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