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SIALIC ACID VALUE IN PLEURAL EFFUSION AS A DIAGNOSTIC MARKER OF MALIGNANCY

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Abstract

In differential diagnosis of pleural effusions, cytology is the most sensitive method. Since cytology findings are positive in half of such fluids, combined use of reliable tumor marker and cytology is a logical approach. Sialic acid and other tumor markers can be reliable substances associated with neoplasia.

The present study measured sialic acid levels in pleural effusion and serum samples of patients with malignant and nonmalignant diseases to discriminate each other, and to diagnose malignant effusion in a simple, cheap and reliable way. Sixty patients with pleural effusion were enrolled in the study and classified into two groups, group (I) 30 patients with malignant pleural effusion, and group (II) 30 patients with nonmalignant pleural effusion. Pleural fluid (PF) and serum (S) levels of sialic acid were measured prior to any therapy; and PF/S sialic acid ratios were calculated.

Pleural fluid and serum levels of sialic acid were significantly higher in malignant group compared to nonmalignant one. PF/S sialic acid ratio was higher in malignant group compared to nonmalignant group. In the malignant pleural effusion group, smokers showed a statistically significant higher pleural fluid and serum sialic acid levels as compared to nonsmokers. By using ROC curve, the cut off value of malignant pleural fluid sialic acid was 69.65 mg/dL, sensitivity was 70%, and specificity was 96%.

Key words: Sialic acid, pleural effusion, lung neoplasms.

Introduction

Pleural effusion is an excessive accumulation of fluid in the pleural space, indicates an imbalance between pleural fluid formation and removal. Pleural effusions accompany a wide variety of disorders of the lung, pleura, and systemic disorders (Vinaya and Jyotsna, 2012).

The most common conditions that result in effusions are cardiac failure, pneumonia, and malignant neoplasm. Malignant disease involving pleura is the second leading cause of exudative pleural effusions after para-pneumonic effusions (Emmet and Paul, 2011).

It is important to elucidate the precise etiology to differentiate benign from malignant effusions. The initial diagnostic approach includes thoracocentesis and cytologic, histologic, and biochemical examinations (Light, 1995).

However, the sensitivity of these non-invasive techniques is only 40%-70% (Marel *et al*, 1995). To improve upon these rates, a number of tumor markers in the pleural fluid have been intensively evaluated and reported to have diagnostic rule or value in malignant effusion (Villena *et al*, 1996).

One of these markers is sialic acid (SA). Sialic acid levels had been found to be elevated in neoplastic cells derived from lung, breast, stomach, colon, ovary, prostate and liver. Recently sialic acid had been reported to have a diagnostic value in malignancy (Nicola and Prasad, 2002).

Sialic acid is the generic term given to a family of acetylated derivatives of neuraminic acid (N-acetyl neuraminic acid; NANA), which occur mainly at terminal positions of glycoprotein and glycolipid oligosaccharide side chains. Several biological functions have been suggested for SA, such as stabilizing the conformation of glycoproteins and cellular membranes, assisting in cell-cell recognition and interaction, contributing to membrane transport, providing binding sites for ligands for the membrane receptor functions, and affecting the function, stability and survival of glycoproteins in blood circulation (Selva and Kalaivani, 2011).

The present study aimed to measure Sialic acid levels in pleural effusion and serum samples of patients with malignant and nonmalignant diseases to discriminate each other, and to diagnose malignant effusion in a simple, cheap and reliable way.

Subjects, Materials and Methods

Sixty patients with pleural effusion were enrolled and classified into two groups as follows:

GI (n=30): patients with malignant pleural effusion (proved malignant by pleural biopsy), they were 11 females and 19 males, 25 with lung cancer, 3 with mesothelioma, 1 with esophageal neoplasia, and 1 with mediastinal neoplasia (age range 25 to 72 years).

GII (n=30): control patients with non-malignant pleural effusion, they were 8 females and 22 males, 20 with tuberculosis, 5 with pneumonia, 3 with cardiac failure, 1 with liver cirrhosis, and 1 with chronic renal failure (age range 26 to 70 years). 16 patients from group (I) were smokers, while 19 patients from group (II) were smokers.

All patients were recruited from the NJCH Outpatients' Clinic of Chest Department, in the years 2012-2013. All participants gave their informed consent before participation in the study, and the Ethics Committee of NJCH Hospital approved the protocol of the study.

They were subjected to detailed clinical history, thorough clinical examination and routine investigation. None of the patients was receiving drugs known to affect the investigated parameter.

Pleural fluid and serum samples were collected from each patient prior to any therapy. The supernatant of pleural fluid samples were obtained by centrifugation at 300 rpm for 15 minutes and stored at -20°C until assayed. 10 ml blood was collected from the antecubital vein, centrifuged at 3,000 rpm for 10 minutes at room temperature; and

serum was stored, in aliquots, at -20°C until used.

Sialic acid levels were estimated by Warren's TBH method (Warren, 1959). A calorimetric assay in which sialic acids are oxidized with sodium periodate in concentrated phosphoric acid. The periodate oxidation product is coupled with thiobarbituric acid and the resulting chromophore is extracted into cyclohexanone. The thiobarbituric acid assay is reproducible, sensitive, and considerably more specific than other methods.

Statistical analysis: The results were organized, tabulated and statistically analyzed using Statistical Package for Social Science (SPSS) program version 10, Chicago-IL, USA (Norusis, 1997). Data were presented as M±SD and percentages when appropriate. Student t-test was used for analysis of two quantitative data. Differences among the two groups were compared by one-way ANOVA followed by post-hoc test. Simple linear correlation (Pearson's correlation for quantitative data and Spearman correlation for qualitative data) was done. ROC (Receiver Operating Characteristic) curve was drawn for detection of reliability of the marker and its best cutoff value.

Specificity and sensitivity were calculated, and AUC (area under the curve) was considered if > 0.60. p value ≤ 0.05 was considered significant.

Results

The main demographic data (M± SD) among the groups are shown in table (1). There was no significant difference between groups as regard age, sex, or smoking.

Table I: Demographic data of groups

Variables	Malignant Pleural Effusion (n= 30)	Non-malignant Pleural Effusion (n=30)	p-value
Age (years)	58.4 ± 13.1	55.3 ± 11.02	0.7
Sex (F/M)	11/19	8/22	0.8
Smoking	16	19	0.8

Values expressed as means ± S.D, P-value ≤ 0.05= significant*

Table 2: Causes of malignant and nonmalignant pleural effusion among groups

Groups	Causes	Number of cases (%)
Malignant pleural effusion (G I, n=30)	lung cancer	25 (83.33%)
	Mesothelioma	3 (10.00%)
	Esophageal neoplasia	1 (03.33%)
	Mediastinal neoplasia	1 (03.33%)
Nonmalignant pleural effusion (GII, n=30)	Tuberculosis	20 (66.66%)
	Pneumonia	5 (16.66%)
	Cardiac failure	3 (10.00%)
	Liver cirrhosis	1 (03.33%)
	Chronic renal failure	1 (03.33%)

Pleural fluid levels of sialic acid were statistically and significantly higher in malignant group compared to nonmalignant one (76.23±11.18 vs. 57.11± 15.22, p< 0.05). Also, serum levels of sialic acid were statistically and significantly higher in malignant group compared to nonmalignant one (85.03±

10.04 vs. 70.53±6.19, p< 0.05). Ratio of sialic acid levels in pleural fluid to serum (PF/S ratio) was higher in malignant group (0.89±0.068) as compared to nonmalignant group (0.81± 0.21), though difference was not statistically significant (Tab. 3).

Table 3: Sialic acid levels among groups

Variable	Malignant Pleural Effusion	Nonmalignant Pleural Effusion	p-value
PF sialic acid (mg/dL)	76.23 ± 11.18	57.11 ± 15.22	0.000*
S sialic acid (mg/dL)	85.03 ± 10.04	70.53 ± 6.19	0.000*
PF/S sialic acid	0.89 ± 0.068	0.81 ± 0.21	0.059

Values expressed as M ± S.D, P-value ≤ 0.05*= significant, PF= Pleural fluid, S=Serum, PF/S= Pleural fluid/serum ratio

In malignant group, smokers showed a statistically significant higher pleural fluid and serum sialic acid levels as compared to nonsmokers (81.00 ± 9.45 vs. 65.11 ± 5.55 and 89.00±8.95 vs. 75.78 ±5.28, respectively) (p= 0.00). While PF/S sialic acid ratio, in smokers compared to nonsmokers of same group, showed higher but not statistically significant value (0.91±0.06 vs. 0.86±0.05) (p< 0.05). In the nonmalignant group (II), no significant change between smokers and nonsmokers was found regarding pleural fluid and serum sialic acid levels and PF/S sialic acid ratio (tab. 4).

Table 4: Sialic acid levels among smokers and nonsmokers groups

Variables	Malignant Pleural Effusion		p-value	Nonmalignant Pleural Effusion		p value
	Smokers	Nonsmokers		Smokers	Nonsmokers	
PF Sialic acid (mg/dL)	81.00 ± 9.45	65.11 ± 5.55	0.000*	56.36 ± 15.36	57.86 ± 15.58	0.793
S Sialic acid (mg/dL)	89.00 ± 8.95	75.78 ± 5.28	0.000*	70.53 ± 5.59	70.53 ± 6.93	1.000
PF/S Sialic acid	0.91 ± 0.06	0.86 ± 0.05	0.061	0.809 ± 0.23	0.824 ± 0.20	0.857

Values expressed as M± S.D, P-value is significant if≤ 0.05*

A statistically significant positive correlation was found between pleural fluid sialic acid and serum sialic acid levels in malignant group (Fig. 1), but no significant correlation could be observed in nonmalignant group.

By using receiver operator characteristic (ROC) analysis, in malignant pleural effusion cut off value of pleural fluid sialic acid was 69.65 mg/dl, sensitivity was 70%, specificity was 96% and positive predictive value (PPV) was 46.34% (Fig. 2, Tab. 5). This approved value of pleural fluid sialic acid as a promising marker for diagnosis of malignant pleural effusion.

Table 5: Sensitivity, Specificity, Best Cutoff value and PPV of pleural fluid Sialic acid

AUC	P value	Best Cutoff	Sensitivity	Specificity	PPV
0.884	< 0.0001*	69.65 (mg/dL)	70%	69%	46.34%

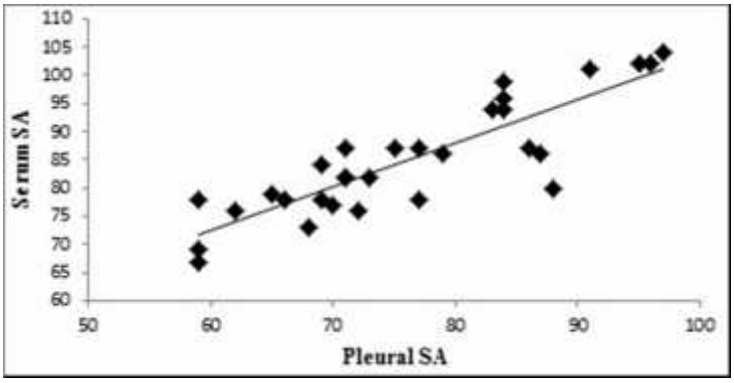


Fig. 1: Correlation between serum sialic acid (SA) and pleural Ssialic acid (SA) among malignant cases

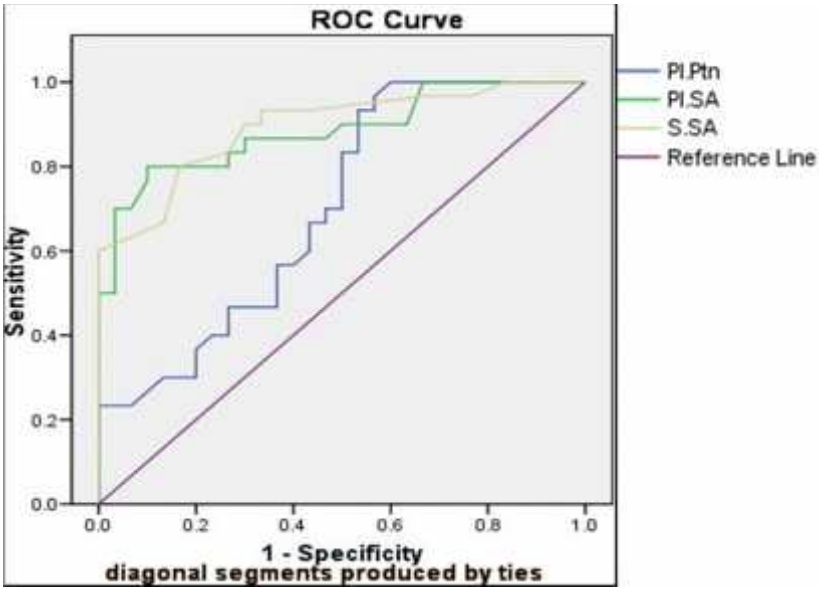
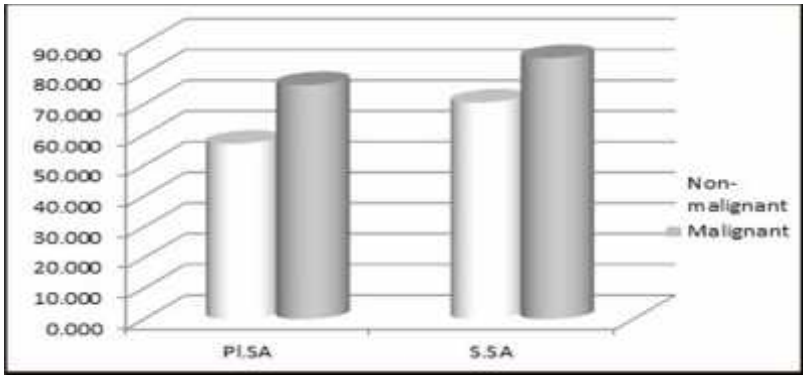


Fig. 2: ROC curve of pleural fluid Sialic acid



Discussion

In differential diagnosis of exudative pleural effusions, cytology is the most sensitive method. Since cytology findings are positive in half of such fluids, combined use of reliable tumor marker and cytology is a logical approach.

The determination of tumor markers in pleural effusions has been proposed as an alternative, noninvasive way of establishing a diagnosis of pleural malignancy. However, these measurements in clinical practice remains controversial (David *et al*, 2005).

Sialic acid and other tumor markers are assuming increasingly important role in clinical oncology, accordingly identification of reliable markers or substances associated with neoplasia is the goal of many researches (Erbil *et al*, 1985).

The present study showed a statistically significant increase in sialic acid levels in both pleural fluids and sera of patients with malignant pleural effusion compared to nonmalignant effusion. Also, PF/S sialic acid ratio was higher in malignant group compared to nonmalignant but with no significant difference. These results agreed with Krolikowski *et al*. (1976) who showed a significant difference in the levels of sialic acid in patients with lung cancer compared to normal controls. Hence they reported sialic acid to be of value as a biologic marker in lung cancer. Seber *et al*. (1989) also showed that sialic acid levels in bronchalveolar lavage fluid were highly correlated with the diagnosis of bronchial cancer. Kakari *et al*. (1991) determined total sialic acid (TSA) and lipid-bound sialic acid (LSA) concentrations in comparison to CEA levels in 152 untreated patients with primary lung cancer and 107 benign pulmonary diseases. They concluded that although TSA and LSA were highly sensitive markers of lung cancer, their specificity was low in this setting. Imecik and Ozer (1992) measured sialic acid levels in the pleural effusions from patients with malignant and benign lung pathologies. Finding higher sialic acid concentrations in malignant pleural effusions than in those of benign origin, they concluded that the sialic acid had diagnostic value. Patel *et al*. (1995) showed that carcinoembriyonic antigen (CEA) and phosphohexose isomerase (PHI) levels along with total sialic acid/total protein ratios (TSA/TP) in 192 untreated lung cancer patients and in 80 age- and sex-matched control cases were compared. PHI and TSA/TP were found to be as an important tumor marker in patients with lung cancer.

Also, the present data agreed with Imecik and Ozer (1992) who found 33.33% of cases of malignant pleural effusions had raised pleural fluid sialic acid as compared to serum levels which were in the normal range. This could be either due to high production and gradual absorption of sialic acid in the diseased area or elevation of both serum and pleural fluid levels, but sialic acid disappears more slowly from pleural fluid. Also, Bektemur *et al*. (2003) reported high serum sialic acid levels in malignant pleural effusions though difference was not statistically significant. They also observed high pleural fluid

sialic acid levels and PF/S sialic acid ratio.

On the contrary, Isitmangil *et al*. (2001) reported that sialic acid levels in bronchoalveolar lavage fluid and serum did not show any statistically significant difference between subjects with malignant and the non-malignant lung diseases ($p > 0.05$). Sialic acid levels were also unrelated to the stage and localization of the tumor ($p > 0.05$). They concluded that sialic acid levels did not appear to be a good marker for discriminating malignant from nonmalignant diseases of the lung.

The current study showed a significant higher pleural fluid and serum sialic acid levels in smokers as compared to nonsmokers of the malignant group ($p< 0.001$), also, the PF/S sialic acid ratio, in smokers compared to nonsmokers of the same group, showed higher but not statistically significant value. While no significant changes were detected for the same parameters in the nonmalignant group between smokers and nonsmokers.

In smokers serum sialic acid increased since smoking induces tissue inflammation in addition of being known as carcinogenic (Crook *et al*, 2000; Kurtul, *et al*, 2004).

By using ROC analysis, the present study found that in malignant pleural effusion the cut off value of pleural fluid sialic acid was 69.65 mg/dL, sensitivity was 70%, and specificity was 96%. This agreed with Bektemur *et al*. (2003) who reported that in differential diagnosis of malignant pleural effusions, sensitivity and specificity of pleural fluid sialic acid were 61%, and 91%, respectively.

Also, Bansal *et al*. (2010) while taking the cut off value of 70 mg/dL for pleural fluid sialic acid in malignant pleural effusions, they found that the sensitivity was 63.33%, specificity 60%, and they concluded that determination of sialic acid levels in pleural fluid has diagnostic value as a cheap, simple and reliable marker for malignant pleural effusion.

Conclusion

The outcome findings suggests that determination of sialic acid levels in pleural fluid has diagnostic value as being cheap, simple and reliable marker for malignant pleural effusion and combined use of sialic acid with cytology is highly recommended for further research evaluation

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