SERUM LIPID-BOUND SIALIC ACID IN LUNG CANCER PATIENTS

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Serum lipid-bound sialic acid (LSA) assay was evaluated for differential diagnosis between benign and malignant pulmonary disease. Mean serum LSA for normal controls was 13.7±3.7 mg/dl and the upper limit of the normal value was set at 19.8 mg/dl (95% confidence limit, one side). The specificity was 97.1% (95% confidence interval was 93.8%, 100.0%). The mean for benign pulmonary disease was 17.1±5.1 mg/dl, the specificity was 89.1% (95% confidence interval was 80.9%, 97.3%), and the sensitivity was 10.9%. The mean for malignant pulmonary disease was 27.1±7.9 mg/dl, and the sensitivity was 93.3% (95% confidence interval was 88.5%, 98.1%). The serum LSA levels for different malignant pulmonary diseases were different between small cell carcinoma and adenocarcinoma. The results indicate that serum LSA assay might be used as tumor marker to differentiate lung cancer from normal subjects or benign lung disease.

Key words: Serum, Lipid-bound sialic acid, Lung cancer, Resorcinol-HCL.

Serum/plasma lipid-bound sialic acid (LSA) was elevated in patients with cancer. LSA assay might be useful in differential diagnosis, staging of disease, detection of metastasis, identifying patients at high risk for recurrence, and evaluating therapeutic response. Combined with other methods, LSA might be used as a tumor marker in cancer screening.

The incidence rate of lung cancer was the highest one among all malignant diseases in Tianjin urban area. In the period of 1981–1982, the age-adjusted incidence rate was 43.0/100,000 for male and 28.8/100,000 for female. Because of the high incidence and poor prognosis for later stage patients with lung cancer, more efforts should be made to develop methods for the early detection of lung cancer.

The purpose of this investigation is to determine if the measurement of serum LSA levels is clinically useful in lung cancer patients. Specificity in normal blood donors and patients with benign pulmonary diseases as control groups and sensitivity in patients with different types of lung cancer were investigated in this study.

MATERIALS AND METHODS

General Data

One hundred and two (54 men, 48 women) healthy blood donors without malignant or other diseases, between 20 to 45 years of age served as normal controls. Fifty five patients with benign pulmonary disease were investigated as pathological controls, which included 20 patients with pulmonary heart disease, 12 with pneumothorax, 12 with emphysema, 6 with bronchiectasis, 3 with emphysema, one with pneumonia and one with chronic trachitis. One hundred and four patients with malignant pulmonary disease were investigated in this study.
including 30 with squamous cell, 21 with adenocarcinoma, 18 with small cell, 11 with undifferentiated cell, 2 with large cell, 2 with carcinosarcoma and 20 unspecified. All patients with malignant disease were pathologically confirmed and untreated by chemotherapy or radiotherapy or operation.

Sera

Sera were separated from clotted blood by centrifugation and were assayed immediately after collection or otherwise, frozen at -20°C until used.

Chemicals

The chemicals used were reagent grade and distilled water was used throughout. The sialic acid (N-acetyl neuraminic acid) at a purity of 95% was the product of Sigma Chemical Company, ST. Louis, MO.

Assay

LSA was determined by the modified resorcinol-HCl method.\(^4\) One hundred μl of serum were placed in 10 ml test tubes (in duplicate) with 150 μl distilled water. The tubes were vortexed for 5 sec and transferred to crushed ice. Three ml of cold (4°C) chloroform/methanol (2:1, v/v) was added to each tube and the tubes were vortexed for 30 sec. To this mixture, 0.5 ml cold distilled water was added, the contents were vortexed for 15 sec and then centrifuged for 5 min at 3000 rpm. One ml of the upper layer was transferred into a 10 ml test tube. Fifty μl phosphotungstic acid solution (0.5 g/ml) were added, and after mixing the tubes stood at room temperature for 3 min. The tubes were centrifuged for 5 min at 2500 rpm, and the supernatant was removed by suction. One ml distilled water was added, and the tubes were vortexed until the precipitate was in suspension. One ml of the resorcinol reagent (10 ml resorcinol + 78 ml HCl + 2 ml 0.1 M FeCl\(_3\), add distilled water to 100 ml) was added, and the tubes were vortexed and placed in a boiling water bath for exactly 15 min. Immediately after the 15 min, the tubes were transferred to an ice and water bath and left for 5 min. To the ice-cold tubes, 2 ml 85:15 (v/v) butyl acetate:n-butyl alcohol were added, and the tubes were vortexed and centrifuged for 5 min at 1500 rpm. The blue colored layer was read at 590 nm and the amount of LSA was determined from a standard curve using authentic n-acetyl neuraminic acid.

Correlation Analysis

It was used to investigate the association between age and serum LSA. One side test was used for estimation of normal value of serum LSA.\(^5\) Sensitivity and specificity were calculated for specified groups.\(^6\) Student's test and Fisher's test were used to compare the differences between groups.\(^5\)

RESULTS

There is no association between age and the level of serum LSA (correlation coefficient=-0.103, \(P=0.3021\)).

Results for serum LSA in normal subjects are presented in Table 1. The average value for 102 healthy blood donors is 13.7±3.7 mg/dl. No significant difference was found between male and female (\(P=0.9228\)). Based on one side estimation of normal value, the upper value of 95% confidence limits is 19.78 mg/dl. Serum LSA values greater than 19.78 mg/dl were considered positive.

Table 1. Serum LSA values by sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Sample size</th>
<th>(\bar{x} \pm s)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>54</td>
<td>13.8±3.7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>48</td>
<td>13.7±3.7</td>
<td>0.9228</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>13.7±3.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows that 3 false positives were found in 102 healthy subjects giving a specificity of 97.1% (95% confidence interval 93.8%, 100.0%). In patients with benign pulmonary diseases, the mean value was 17.1±5.1 mg/dl and the specificity was 89.1% (95% confidence interval 80.9%, 97.3%).

The average value of serum LSA for patients with malignant pulmonary diseases was 27.1±7.9 mg/dl and statistically different from normal control (\(P<0.0001\)). The sensitivity for this group was 93.3% (95% confidence interval was 88.5%, 98.1%). (Table 3 and Figure 1). For different types of malignant pulmonary diseases, small cell had the highest level of serum LSA, which was 31.7±10.9 mg/dl and