Serum Free and Bound Sialic Acid and Alpha-1-Acid Glycoprotein in Patients with Laryngeal Cancer

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Abstract. Serum specimens from 35 patients with laryngeal cancer (10 at stage II; 12 at stage III; 13 at stage IV) were obtained before therapy was initiated. Concentrations of bound sialic acid (BSA), free sialic acid (FSA), and α-1-acid glycoprotein (AAG) were compared to those in serum specimens from 34 healthy controls. The mean levels of serum BSA and AAG were significantly increased in the laryngeal cancer patients versus the controls; there was no significant difference in serum FSA levels between the patients and controls. Mean serum BSA and AAG levels were lowest in the control group and highest in the stage IV patients. As the stage of the cancer advanced, progressively higher levels of serum BSA and AAG were observed. The results indicate that serum BSA and AAG, but not FSA, show correlation with the stage of laryngeal carcinoma. (received 23 September 2002; accepted 26 November 2002)

Keywords: α-1-acid glycoprotein, free and bound sialic acid, laryngeal cancer, acute phase reactants

Introduction

Cancer of the larynx is fourteenth most common cancer in the world. In 1996, an estimated 190,000 new cases of laryngeal cancer were diagnosed worldwide, accounting for 1.8% of all new cancers [1].

Sialic acids (SAs) are derived from neuraminic acid by substitution for an amino group by either an acetyl or glycolyl group. The main derivative is N-acetylneuraminic acid, which is generally used as a synonym for sialic acid [2]. SAs are widely distributed in nature as non-reducing termini of glycoproteins and glycolipids. About 70% of the total sialic acid (TSA) of eukaryotic cells is found on the cell surface and the remainder is distributed primarily in the endoplasmic reticulum, mitochondria, and lysosomes [3]. Because of their acidic nature, SAs impart a negative charge to the cell surface and are important in cell-to-cell or cell-to-matrix interactions. SA residues on the cell surface may also be involved in masking cell surface antigens and may serve as receptors for lectins, virus particles, some hormones, and antibodies [4].

The surface properties of tumor cells differ from their normal counterparts, owing in part to altered sialoglyco-conjugates that are expressed on the plasma membrane [5]. Although elevated levels of SA have been associated with malignancy [6], a clear correlation of changes in SA concentrations and malignancy has not emerged. Some reports show a decrease and not an increase in SA in association with malignancy [7]. Evaluation of SA changes might contribute to diagnosis of cancer patients and to monitoring the tumor progression and response to treatment [8].

In human serum, SA is present in α-1-acid glycoprotein (AAG), haptoglobin, ceruloplasmin, and transferrin, which are acute phase reactants [9,10]. The serum concentration of AAG, a 44 kDa protein, increases approximately 2- to 4-fold following tissue injury. It has been speculated that AAG plays a key role in inflammation, and may be a good parameter for pre-therapeutic prognostic evaluation of lung cancer patients [11,12].

To our knowledge, there are no previous reports of serum levels of bound sialic acid (BSA), free sialic
acid (FSA), and AAG in laryngeal cancer. In the present study, we investigated serum BSA, FSA, and AAG levels in patients with laryngeal cancer and examined the correlation of serum BSA, FSA, and AAG levels with the cancer stage.

Materials and Methods

Patients. Thirty-five men with laryngeal cancer comprised the patient group and 34 healthy men comprised the control group. Their age was 37 to 63 yr (54.2 ± 8.1 yr) for the laryngeal cancer group and 35 to 58 (50.3 ± 6.3 yr) for the controls. The cancer patients and controls were all smokers, but not drinkers. The American Joint Committee on Cancer Tumor (AJCC) staging schema, based on TNM (T tumor size; N node invasion; M metastasis) was applied [13]. The laryngeal cancer patients included 10 cases at stage II (T2N0M0), 12 cases at stage III (T2, T3N1M0, or T3N0M0), and 13 cases at stage IV (T3, T4N1M0, T4N2M0, T4N0M0, or T4N3). Twelve patients were T2; 12 were T3; 11 were T4. No lymph node metastasis was evident in 22 patients. Ten patients were N1; 2 were N2; 1 was N3. None of the patients had distant metastases. The patients and healthy controls were enrolled in the study after giving their informed consent. Venous blood samples (8 to 10 ml) were centrifuged and the serums were stored at -80°C until analysis.

Biochemical measurements. FSA was determined by the thiobarbituric acid method of Aminoff [14]. Briefly, 0.5 ml of serum was mixed with 0.25 ml of 25 mM periodic acid in 0.125 N H2SO4 (pH 1.2) and heated for 30 min in a water bath at 37 °C. Excess periodate was then reduced with 0.2 ml of 2% (w/v) sodium arsenite in 0.5 N HCl. As soon as the yellow color of liberated iodine disappeared (1-2 min), 2 ml of 0.1 M thiobarbituric acid solution was added. The tube was capped and heated in a boiling-water bath for 7.5 min. The colored solution was cooled in ice-water and extracted with 5 ml of acid butanol (butan-1-ol containing 5% (v/v) of 12 N HCl). After centrifugation at 3000 x g for 5 min, the absorbance of the butanol extract was measured at 549 nm using a CE 3041 spectrophotometer (Cecil, Ltd., Cambridge, UK). Serum TSA was quantified by the same procedure after hydrolysis of the sample in 5 vol of 0.1 N H2SO4 at 80°C for 1 hr. TSA and FSA concentrations of serum samples were calculated from a standard calibration curve and BSA was determined as the difference between TSA and FSA. Serum AAG level was assayed by a nephelometric method (Beckman Array 360 System, Beckman-Coulter Corp, Brea, CA).

Statistical analysis. Results were expressed as mean ± SD. Statistical and correlation analyses were performed by the Mann-Whitney U-test and Spearman’s rank correlation test, respectively, using the Statistical Package for the Social Sciences (version 10.0 for MS Windows, SPSS, Inc., Chicago, IL, USA); p <0.05 was considered significant.

Results

As listed in Table 1, the mean serum BSA and AAG levels were lowest in the controls and highest in the stage IV patients with laryngeal cancer. In all of the patient groups (stages II, III, IV), the mean serum

Table 1. Serum levels of BSA, FSA, and AAG in healthy controls and patients with laryngeal cancer (means ± SD).

<table>
<thead>
<tr>
<th>Category</th>
<th>Serum BSA (mg/L)</th>
<th>Serum FSA (mg/L)</th>
<th>Serum AAG (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects (n = 34)</td>
<td>502.6 ± 89.5</td>
<td>15.9 ± 5.5</td>
<td>846 ± 127.8</td>
</tr>
<tr>
<td>Laryngeal cancer patients</td>
<td></td>
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<td></td>
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<tr>
<td>Stage II (n = 10)</td>
<td>649.0 ± 141.8b</td>
<td>16.2 ± 4.9</td>
<td>1103.0 ± 134.8a</td>
</tr>
<tr>
<td>Stage III (n = 12)</td>
<td>790.7 ± 168.7a,c</td>
<td>16.5 ± 3.6</td>
<td>1383.8 ± 136.8a,d</td>
</tr>
<tr>
<td>Stage IV (n = 13)</td>
<td>911.2 ± 151.2a,d</td>
<td>17.5 ± 4.2</td>
<td>1696.7 ± 149.6a,d,e</td>
</tr>
<tr>
<td>Patients without cervical metastases (N0, n = 22)</td>
<td>692.9 ± 126.7</td>
<td>16.9 ± 4.5</td>
<td>1263.6 ± 227.6</td>
</tr>
<tr>
<td>Patients with cervical node metastases (N+, n = 13)</td>
<td>794.9 ± 184.5</td>
<td>16.8 ± 4.2</td>
<td>1642.0 ± 193.8f</td>
</tr>
</tbody>
</table>

a p <0.001, b p<0.01 vs controls; c p <0.05, d p< 0.001 vs stage II; e p <0.001 vs stage III; f p <0.01 vs N0 group.
BSA and AAG levels were significantly higher than in the controls. Moreover, mean serum BSA and AAG levels increased progressively in stages II, III, and IV. The mean serum FSA level in controls did not differ significantly from the levels in the patient groups.

In 13 patients with metastatic laryngeal cancer in cervical lymph nodes (N1), the mean serum AAG level was significantly higher than in the 22 patients without such metastases (N0).

As shown in Fig. 1, positive correlations were observed between serum BSA and AAG levels in the laryngeal cancer patients at stages II, II, and IV (stage II, r = 0.73, p <0.05; stage III, r = 0.69, p <0.05; stage IV, r = 0.88, p <0.001). These correlations became more significant as the stage of the disease increased.

Discussion

The surface glycoproteins and glycolipids of tumor cells have altered carbohydrate composition, which may contribute to the aberrant cell-cell recognition, cell adhesion, antigenicity and invasiveness of malignant cells. SAs are major constituents of glycoproteins and glycolipids, and studies have shown that serum levels of total bound sialic acid or protein-bound SA are higher in patients with cancer as compared with normal subjects [15]. Lipid-bound SA levels are correlated with the stage of the disease, degree of metastatic involvement, and recurrence of disease [15]. AAG is one of the acute phase proteins and its serum concentration in plasma increases approximately 2- to 4-fold following tissue injury. It has been speculated that AAG plays an important role in inflammation and cancer, but its biological functions are still unclear [16].

The present study demonstrates that BSA and AAG levels are higher in patients with laryngeal cancer, compared to healthy controls. However, the serum FSA levels did not differ significantly in the patients with laryngeal cancer, compared to controls. This latter result is in agreement with the results of a previous study [15].

Clinical and laboratory studies have shown that neoplastic transformation leads to elevated serum SA concentration. TSA and BSA levels higher than those for control subjects have been reported in serum from patients with various malignant diseases, including colorectal, head and neck, and breast cancers; markedly increased TSA levels were observed in patients with more advanced malignancies [15,17-19]. Romppanen et al [20] reported that patients with malignant breast disease had the highest serum TSA levels, those with benign breast disease had intermediate levels, and healthy control women had the lowest levels. In a previous study, we observed increased serum TSA levels in patients with laryngeal cancer [21].

The mechanism of increased serum SA concentration in malignancies and inflammatory conditions is unclear, but several explanations have been proposed. These include: (a) spontaneous release of aberrant SA-containing cell surface glycoconjugates, (b) increased concentrations and/or glycosylation of normal serum glycoproteins, and (c) secondary inflammatory reactions leading to increased hepatic output of acute phase proteins [22,23]. Moreover, increased activity of serum sialyltransferase was detected in some cultured cancer cells that secrete glycoproteins into the growth medium. Tumor cells also secrete their membrane constituents into intracellular fluid. These processes of secretion may partly explain the increased serum
SA concentration in cancer [24,25]. Why FSA is not increased in cancer is a point that deserves further investigation.

In conclusion, increased serum BSA and AAG levels are associated with laryngeal cancer and appear to be a consequence of the disease itself. These molecules and other acute phase reactants may play important metabolic roles in cancer progression.

References