The Significance of Serum Soluble Intercellular Adhesion Molecule 1 and Transforming Growth Factor α in Patients With Nasopharyngeal Carcinoma

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Objective: To determine serum levels of soluble intercellular adhesion molecule 1 (sICAM-1) and transforming growth factor α (TGF-α) in 51 patients with nasopharyngeal carcinoma before, during, and after radiation therapy (5-year follow-up period).

Results: The mean±SD serum levels of the 2 cytokines were found to be higher in patients before radiotherapy (sICAM-1, 369.6±123.7 ng/mL; TGF-α, 36.6±24.6 ng/mL) than after radiotherapy (sICAM-1, 225.9±124.3 ng/mL; TGF-α, 20.2±22.3 ng/mL) (P<.05), and they were significantly higher in patients with recurrence (sICAM-1, 512.5±271.2 ng/mL; TGF-α, 48.2±23.4 ng/mL) and in those who died (sICAM-1, 542.6±245.4 ng/mL; TGF-α, 50.2±28.8 ng/mL) than in patients with no recurrence (sICAM-1, 217.9±116.4 ng/mL; TGF-d, 21.5±26.8 ng/mL) and in those who survived (sICAM-1, 209.4±167.2 ng/mL; TGF-α, 20.4±28.8 ng/mL) (P<.05). The increases in serum levels occurred approximately 3 months before relapse.

Conclusion: We found that sICAM-1 and TGF-α levels are extremely useful markers for predicting illness, recurrence, and survival.

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One of the most important problems in the treatment of nasopharyngeal carcinoma (NPC) is recurrence. One reason for recurrence is that local deep infiltration or distant blood-borne metastasis can develop insidiously, perhaps even before “curative” treatment is undertaken. Tumor growth and the formation of metastases represent a multistage process involving complex interactions between tumor cells and the vascular endothelium.1 Cellular adhesion molecules play an important role in the process of metastasis. Positive and negative regulation of cell adhesion will influence the process as metastatic cells break away from the primary tumor, travel in the circulation, and then adhere to cellular and extracellular matrix elements in some secondary sites. Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily of adhesion molecules.2 It is an inducible ligand for lymphocyte function–associated antigen 1 (LFA-1) and heavily influences cell-to-cell interactions in inflammatory and immune responses.3 This interaction is also implicated in the various stages of tumor progression and metastasis.4 A soluble form of ICAM-1 (sICAM-1) has been found in serum samples from normal subjects5 and in those from patients with malignancies6; it can be released by cancer cells7 as well as by peripheral blood mononuclear cells, endothelial cells, and fibroblastic cells.8 Proteolytic cleavage of membrane-bound ICAM-1 may be the most likely mechanism for the generation of sICAM-1.9 The significance of adhesion molecule shedding is not clear, but it may have profound implications for tumor metastasis. Shedding of ICAM-1 by circulating tumor cells may allow their escape from surveillance by cytotoxic T and natural killer cells and thus promote metastasis.6 It is possible for sICAM-1 to bind to the LFA-1 molecules of leukocytes and inhibit the binding of cell-surface ICAM-1 on cancer cells with leukocytes.10,11 Therefore, the production of sICAM-1 has been thought to play a role in avoiding ICAM-1/LFA-1–mediated tumor cell cytotoxicity.

Transforming growth factor α (TGF-α) has been shown to be an important proliferation activator in various types of epithelial tissue.12 It is a potent mitogenic polypeptide13 and is synthesized as a 160–amino acid transmembrane precursor. Mature TGF-α is a 30–amino acid polypeptide of 5500 Da.14,15 It is secreted by a
variety of transformed cells and tumors, modifying tumor growth through autocrine and paracrine mechanisms. The biologic action of TGF-α is mediated through binding to the cell membrane–bound epidermal growth factor (EGF) receptor, which increases its tyrosine activity and triggers the mitogenic signal transduction. Transforming growth factor α stimulates DNA synthesis in a variety of malignant cells. Transgenic mice that express TGF-α have been reported to complicate carcinogenesis and progression of disease.

However, little is known about serum levels of sICAM-1 and TGF-α in patients with NPC. We used the enzyme-linked immunosorbent assay system to quantify the 2 oncoproteins in serum samples from patients with NPC. The present study was designed to investigate the serum levels of the 2 soluble molecules in patients at presentation, after radiotherapy, and during the follow-up period, as well as to evaluate its clinical significance in association with histologic differentiation, treatment, and tumor recurrence. The levels were measured every 3 months over a 5-year follow-up period.

Initially, 53 patients with NPC without metastasis were included in our study. All patients had stage II disease, and they received radiation therapy (cyanocobalamin Co 60 at a dosage of 60-80 Gy for 6-8 weeks) until clinical healing occurred. The therapy was discontinued in 1 patient halfway through the study because of serious adverse effects, and 1 patient was unavailable for follow-up. Therefore, the study group comprised 51 patients (30 men and 21 women; mean age, 49 years). The age- and sex-matched control group consisted of 64 healthy subjects (45 men and 19 women; mean age, 53 years). Serum samples were obtained at the time of diagnosis, after therapy, and every 3 months during the follow-up period. They were collected and stored at −70°C. Commercially available enzyme-linked immunosorbent assay kits were used to measure the serum levels of sICAM-1 and TGF-α according to the manufacturers’ instructions. Statistical analysis was performed with the t test; a P value of less than .05 was considered statistically significant.

At the end of the follow-up period (January 2003), we conducted a retrospective analysis of the data. Local recurrence and neck lymph node metastasis developed in 9 patients; local recurrence and lung metastasis developed in 8 patients; bone metastasis developed in 5 patients; and brain metastasis developed in 5 patients; 24 of these 27 patients died. Histologic examination showed keratinizing squamous cell cancer (type 1) in 12 patients, nonkeratinizing squamous cancer (type 2) in 28 patients (type 2a, 12 patients; type 2b, 16 patients), and undifferentiated cancer (type 3) in 11 patients.

The mean ± SD serum concentrations of sICAM-1 and TGF-α detected in the control group were 174.6 ± 132.4 ng/mL and 15.3 ± 21.8 ng/L, respectively. Other results are given in Table 1. The mean serum sICAM-1 and TGF-α levels in the patient group before radiation therapy were significantly higher than those 3 months after therapy (P<.03, P<.02), and both levels were significantly higher than those in the control group (P<.02, P<.01). There was no significant difference between the control group and the patient group after 3 months of therapy (P=.06, P=.07).

The serum levels of sICAM-1 and TGF-α were detected every month during follow-up (Figure 1 and Figure 2). There was a good correlation between the 2 cytokines and recurrence (death); the levels of the 2 cyto-

Table 1. Results and Clinical Features

<table>
<thead>
<tr>
<th>No.</th>
<th>sICAM-1, Mean ± SD Level, ng/mL</th>
<th>TGF-α, Mean ± SD Level, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before radiotherapy</td>
<td>51</td>
<td>369.6 ± 123.7</td>
</tr>
<tr>
<td>3 mo after radiotherapy</td>
<td>51</td>
<td>225.9 ± 124.3</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>236.6 ± 115.8</td>
</tr>
<tr>
<td>2a</td>
<td>12</td>
<td>284.6 ± 168.8</td>
</tr>
<tr>
<td>2b</td>
<td>16</td>
<td>324.6 ± 137.3</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>452.8 ± 146.3</td>
</tr>
<tr>
<td>Recurrence group</td>
<td>27</td>
<td>512.5 ± 271.2</td>
</tr>
<tr>
<td>No recurrence group</td>
<td>24</td>
<td>217.9 ± 116.4</td>
</tr>
<tr>
<td>Death group</td>
<td>24</td>
<td>542.6 ± 245.4</td>
</tr>
<tr>
<td>Survival group</td>
<td>27</td>
<td>209.4 ± 167.2</td>
</tr>
</tbody>
</table>

Abbreviations: sICAM, soluble intercellular adhesion molecule 1; TGF-α, transforming growth factor α.

Figure 1. The variation curve of soluble intercellular adhesion molecule 1 (sICAM-1).

Figure 2. The variation curve of transforming growth factor α (TGF-α).
tokines were higher in the recurrence (death) group than those in the group with no recurrence (survival).

The relationship between serum levels of the 2 cytokines and clinical variables is also shown in Table 1. The mean serum sICAM-1 level in the type 1 group was significantly lower than that in the type 3 group (P = .03); there was no significant difference in the serum levels among other histologic types (P > .05). No significant difference in the levels of serum TGF-α was found within the 4 histologic types (P > .05).

The mean serum sICAM-1 and TGF-α levels were higher in the recurrence and death groups than in the no recurrence and survival groups (P = .04; P = .02; P = .03; P = .03; P = .02). Interestingly, a remarkably high serum level was observed 3 months earlier than the recurrence itself. In the recurrence group, the difference in cytokine levels among 4 histologic subtypes was not significant (P > .05) (Table 2). Linear correlation analysis showed significant positive correlations between levels of sICAM-1 and TGF-α (r = 0.71; P = .02).

**Table 2. Cytokine Serum Levels in Recurrence Group by Histologic Subtype**

<table>
<thead>
<tr>
<th>Histologic Subtype</th>
<th>Mean ± SD Level, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sICAM-1</td>
</tr>
<tr>
<td>1 (n = 5)</td>
<td>498.0 ± 207.6</td>
</tr>
<tr>
<td>2a (n = 5)</td>
<td>522.5 ± 231.3</td>
</tr>
<tr>
<td>2b (n = 8)</td>
<td>499.0 ± 241.3</td>
</tr>
<tr>
<td>3 (n = 9)</td>
<td>510.7 ± 243.3</td>
</tr>
</tbody>
</table>

Abbriviations: sICAM, soluble intercellular adhesion molecule 1; TGF-α, transforming growth factor α.

This study describes serial measurements of sICAM-1 and TGF-α levels in serum samples from patients with NPC over a 5-year follow-up period. We observed that serum sICAM-1 and TGF-α levels at presentation are higher in patients with NPC than in healthy controls and that both cytokine levels are significantly associated with radiation therapy. The mean serum levels decreased significantly after radiation therapy. These results suggest that the cancerous tissue might be the source of marked increases in serum levels of sICAM-1 and TGF-α in patients with NPC. The serum levels were significantly higher in the group of patients who developed recurrence (or died) during follow-up. More importantly, significantly high serum levels were observed 3 months earlier than the recurrence itself.

The mechanism by which human cells generate sICAM-1 is not known. However, it has been demonstrated that sICAM-1 may be produced by the proteolytic cleavage of membrane-associated ICAM-1; although sICAM-1 may lack the intercellular and transmembrane region required for cell anchorage, it may possess most of the necessary extracellular structure to retain the functional activities of ICAM-1. TGF-β has been shown to produce ICAM-1 and other adhesion molecules in vitro. TGF-β stimulates cell adhesion through ICAM-1, which is an inducible cell-surface adhesion molecule, and the ICAM-1/LFA-1 pathway plays a major role in a variety of cell-mediated immune responses.

The adhesion molecules that mediate adhesive interactions have recently drawn considerable attention. Neoplastic transformation and the evolution to metastatic disease are characterized by a dramatic aberration in cellular cohesive interaction. The adhesion molecules have also been shown to facilitate tumor cell mobility, adhesion of tumor cells to endothelium, neovascularization at the metastatic sites, and host inflammatory response to cancer. Intercellular adhesion molecule 1 is an inducible cell-surface adhesion molecule, and the ICAM-1/LFA-1 pathway plays a major role in a variety of cell-mediated immune responses. Recently, it has been suggested that this molecule plays a role in the progression of metastasis of some cancers.

Benekli et al reported that serum sICAM-1 levels were significantly increased in 27 patients with gastric cancer compared with 18 healthy controls. Yoo et al studied serum samples from 142 patients with gastric cancer. Among inoperable cancer cases, the sICAM-1 levels were significantly greater in 12 patients with liver metastasis than in 35 patients without liver metastasis.

Transforming growth factor α is a polypeptide that stimulates the growth of various human epithelial cells. Recent studies indicate that the presence of TGF-α is associated with the neoplastic process. Furthermore, TGF-α expression has been found to correlate with poor prognosis. Increased levels of TGF-α have been detected in samples of urine and plasma from patients with cancer, and some authors have focused on the analysis of the parameters of the EGF system, especially TGF-α, EGF receptor: this system is considered to be one of the early responses of the EGF signal transduction pathway. To our knowledge, no studies involving long-term follow-up of serum sICAM-1 and TGF-α levels in NPC have been published to date. The paucity of such information prompted us to perform a retrospective analysis of serum sICAM-1 and TGF-α levels in patients with NPC.

**COMMENT**

The adhesion molecules mediate interactions involving tumor cells, endothelium, and host immune cells. Understanding the mechanisms of adhesion is crucial for the development of new therapeutic strategies to prevent metastasis and improve patient outcomes.
might be useful as a potential biomarkers for pre-
therapy diagnosis or follow-up in the management of NPC.

Because we collected a sufficient number of cases and monitored serum levels throughout the follow-up periods, we believe that the results of our study are reliable. The data showed that the levels of the 2 cytokines had a close relationship to NPC and that they were potential indexes of recurrence (death) and therapy effect. In some tumors, cancer cells with a strong capability to secrete more sICAM-1 and TGF-α can block immunologic reaction (sICAM-1); in others, some protooncogene is activated, the tumor cells independent growth (TGF-α) is accelerated, and local deep infiltration or inidious blood-borne metastasis develops. In such cases, conventional radiation therapy would not be effective enough, and the patients might have a recurrence or die.

The present study included a homogeneous population of 51 patients with stage II squamous cell carcinoma treated with the same kind of radiation therapy. We are aware that the tumor N stage before therapy has a prognostic impact; therefore, to determine whether serum levels of sICAM-1 and TGF-α are independent prognostic factors, the criterion for inclusion in this study was absence of metastasis. The 2 cytokines were the performer and the carrier of biologic behavior of cancer; so the manifestation of disease severity as assessed by the determination of their levels should be more objective and accurate. Clinically, the high levels of sICAM-1 and TGF-α were important indicators of the severity of disease and the probability of relapse (death).

In the metastatic mechanism of NPC, the 2 cytokines were crux links; they were predictive markers reflecting the biologic behavior of cancer. The simultaneous measurement of sICAM-1 and TGF-α can provide useful reference data: the higher the levels, the more severe the illness. The analysis and multiple uses of the resources should be extremely helpful in the determination of treatment.

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