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Serum Levels of Interleukin-6 in Patients with Primary Head and Neck Squamous Cell Carcinoma

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Abstract. *Interleukin (IL)-6 plays a central role as a differentiation and growth factor of tumor cells. IL-6 has been identified in a wide variety of malignancies, including head and neck squamous cell carcinomas (HNSCC). The aim of this study was to investigate the association between the serum levels of IL-6 in HNSCC patients and the biological characteristics of the tumor as well as the clinicopathological status of the patients. The circulating level of IL-6 in sera from patients with various HNSCC (n=90) as well as from healthy normal controls (n=39) was investigated. Serum IL-6 concentrations were determined as serum immunoreactivity using a quantitative sandwich enzyme immunoassay technique. For statistical analysis, the Kruskal-Wallis test was performed. The majority of the patients with HNSCC were found to have high serum IL-6 concentrations. The IL-6 levels in the sera of patients with cancer ranged from below the detection limit to 312.8 pg/ml (mean, 19.5 pg/ml). In contrast, the IL-6 serum levels in 39 healthy individuals ranged from below the detection limit to 52.2 pg/ml (mean, 6.0 pg/ml), with the concentration being significantly higher in HNSCC patients (p<0.001). Furthermore, the correlation of the IL-6 serum concentration with tumor stage was significant (p=0.04). Accordingly, there was a significant difference of IL-6 serum concentration of tumors with positive and negative lymph nodes (p=0.045), with concentration being significantly higher in lymph node-positive tumors. Our data on elevated IL-6 serum levels in the majority of HNSCC cancer patients and its correlation with tumor stage and lymph node status suggest that serum IL-6 reflects the proliferative activity of the tumor in patients with head and neck cancer. IL-6 serum determinations might serve as a biological marker and help to identify advanced head and neck tumors.*

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Head and neck squamous cell carcinoma (HNSCC) is an aggressive epithelial malignancy and is the most common neoplasm arising in the upper aerodigestive tract. Currently, approximately 50,000 new cases of HNSCC are reported annually in the United States, and more than 500,000 cases are diagnosed every year worldwide (1), making head and neck cancer a great public health problem. The prevalence of HNSCC is increasing world-wide (2). Despite improvements in locoregional control, morbidity and mortality rates have improved only slightly during the past three decades (3). Therefore, early detection or prevention of this disease is likely to be most effective. The absence of definite early warning signs for most head and neck cancers suggests that sensitive and specific biomarkers are likely to be important in screening high-risk patients. A number of molecular markers have been used to detect these tumors with varying degrees of specificity and sensitivity. DNA markers include TP53, microsatellite instability, and the presence of the human papillomavirus, and the Epstein-Barr virus genomic sequences (4). None of these markers has been shown to universally identify HNSCC. Since the release of cytokines by tumor cells has been reported, the serum levels of these molecules may be useful as additional cancer markers (5).

Interleukin (IL)-6, a multi-functional cytokine, plays a central role as a differentiation and growth factor for a variety of different cells, such as hematopoietic precursor cells, B-cells, T-cells, keratinocytes, neuronal cells, osteoclasts and endothelial cells (6). Several human tumor cell lines, including esophageal squamous cell carcinoma (7), multiple myeloma (8), renal cell carcinoma (9), glioblastoma (10) and lung carcinoma (11), have been reported to produce IL-6. The IL-6-IL-6 receptor autocrine loop has been found to exist in several tumors, including esophageal carcinoma (7), renal cell carcinoma (9) and multiple myeloma (8). In an *in vitro* study, the proliferative

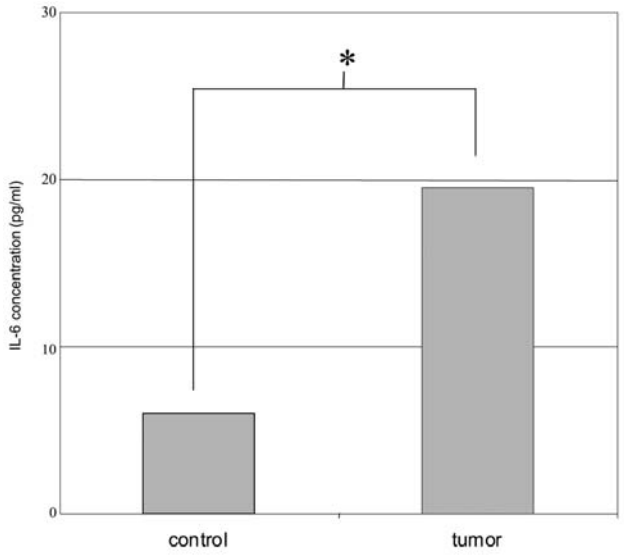

Figure 1. *Comparison of the mean IL-6 serum concentrations in patients with primary head and neck squamous cell carcinoma (HNSCC) (n=90) and healthy controls (n=39), the difference is statistically significant (*).*

response in multiple myeloma cells was increased by IL-6 supplementation (12). In addition, IL-6 was found to rescue resting mouse T-cells from apoptosis (13). These findings suggest that IL-6 produced by the tumor cells acts as a growth factor that interacts with specific membrane receptors on the tumor cell surface to induce proliferation or prolongation of survival in the tumor cells (7).

Although the serum levels of IL-6 have been reported to increase and to correlate with prognosis in patients with various malignant diseases (6, 14-17), the mechanism by which serum IL-6 reflects the disease status is not known. It may be hypothesized that increased serum IL-6 in cancer patients results from overproduction in the tumor, and consequently serum IL-6 reflects the biological characteristics of the tumor. However, the role of IL-6 on the growth of head and neck cancer is still poorly understood. Little is also known about its behavior at a circulating level. In this study, IL-6 concentrations in sera from HNSCC cancer patients as well as from healthy controls were measured and were compared with the clinicopathological parameters of the tumor.

Materials and Methods

The circulating levels of VEGF were investigated in sera from 90 patients (13 females, 77 males, age range, 39 to 83; mean age, 59) with histologically diagnosed HNSCC. Eighty-three of the patients had a history of tobacco abuse. For controls, sera were collected from 39 healthy volunteers without history of any known neoplasm, without recent trauma or surgery, and who were not pregnant. All studies were approved by the Ethical Commitee of the Faculty of Medicine Mannheim, University of Heidelberg, Germany. Informed consent was obtained from all participants. Peripheral venous blood samples were taken and collected in sterile test tubes, centrifuged at 1,000 g for 15 min and stored at –20˚C until used.

Serum IL-6 concentrations were determined as serum immunoreactivity by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique (R&D Systems, Wiesbaden, Germany). The system used a solid-phase monoclonal antibody and an enzyme-linked polyclonal antibody raised against recombinant IL-6. According to the manufacturer's directions, each ELISA assay measured 100 µl serum. All analyses and calibrations were carried out in a duplicate. The calibrations on each microtiter plate included recombinant human VEGF standards. Optical density was determined using a microtiter plate reader (Dynatech) at 450 nm. Wavelength correction was set to 540 nm, and concentrations were reported as pg/ml. Serum IL-6 concentrations were determined without knowledge of the main clinicopathological features of the patients studied.

Table I. *Concentrations of IL-6 in the sera of patients (n=90) with squamous cell carcinomas of the head and neck (concentrations demonstrated in pg/ml)*

	S-IL-6 mean	S- IL-6 median	Standard division	Statistical analysis
Tumor Localization				$p=0.3047$
Oral cavity	2.2	2	0.92	
Oropharynx	10.42	5.12	10.67	
Larynx	18.24	9.94	27.91	
Hypopharynx	19.03	12.81	19.81	
Grading				$p=0.4944$
G 1	10.7	10.7	11.3	
G 2	20.49	9.15	41.87	
G 3	19.03	12.92	21.72	
Stage				$p=0.0398$
I	33.13	5.26	65.62	
II	33	4.68	75.42	
III	26.5	3.02	61.79	
IV	47.53	12.28	103.35	
T-stage				$p=0.2113$
T 1	12.73	7	13.16	
T 2	12.3	5.63	14.32	
T 3	11.61	10.32	8.57	
T 4	29.95	12.41	56.37	
Lymph node				$p=0.0452$
Positive	25.05	12.16	47.94	
Negative	12.31	6.92	17.36	

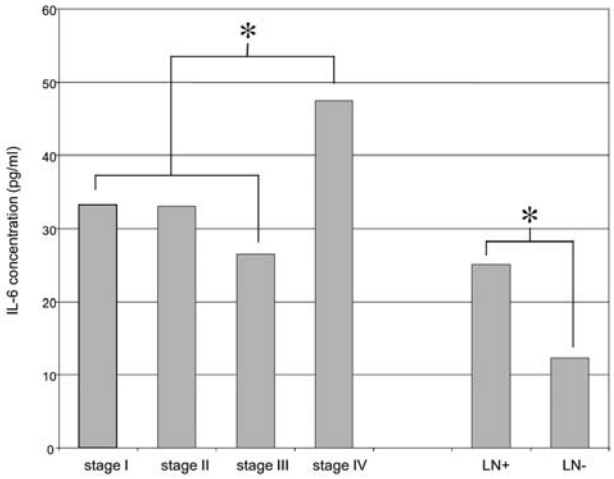

Figure 2. *Mean serum IL-6 concentrations depending on the tumor stage and lymph node status of the HNSCC tumors studied (LN=lymph node; *=statistically significant).*

For statistical analysis, IL-6 serum distributions between the HNSCC cancer patients and normal healthy controls were compared using the Wilcoxon 2-sample test. Differences in serum IL-6 levels were analyzed depending on the main clinicopathological features of the patients (tumor localization, histological grading, T-stage and lymph node status). Serum levels of IL-6 were expressed as mean±standard deviation (SD). All data were subjected to the Kruskal-Wallis test (Chi-square approximation). A p-value <0.05 was considered statistically significant.

Results

When performing the ELISA assay, we found that the majority of the patients with HNSCC had high concentrations of serum IL-6. The levels of IL-6 in the sera of patients with cancer ranged from below the detection limit to 312.8 pg/ml (mean, 19.5 pg/ml). In contrast, the IL-6 serum levels in 39 healthy individuals ranged from below the detection limit to 52.2 pg/ml (mean, 6.0 pg/ml). Thus, IL-6 serum concentrations were significantly higher in the HNSCC patients ($p \leq 0.001$) (Figure 1).

Circulating IL-6 concentrations in relation to the tumor localization, TNM-stage and histological grading of the HNSCC tumors are depicted in Table I. Elevated IL-6 serum levels were measured independently of tumor localization ($p=0.305$), the T-stage ($p=0.212$) or the histological grading of the tumors.

In contrast, the correlation of the IL-6 serum concentration with the tumor stage was significant ($p=0.04$). Accordingly, there was a significant difference of IL-6 serum concentration of tumors with positive and negative lymph nodes ($p=0.045$), the concentration being significantly higher in positive lymph node tumors (Figure 2).

Discussion

The present study demonstrated elevated serum levels of IL-6 in the majority of patients with HNSCC compared to healthy controls. Within the tumor patient cohort, IL-6 serum concentrations were statistically increased in those patients with advanced stage tumors and with a positive cervical lymph node status. Our findings are in line with previous studies that have supported a role for IL-6 in HNSCC and that have demonstrated that IL-6 is detectable at higher concentrations in the serum of patients with HNSCC compared with age-matched control subjects (18, 19, 20).

Accordingly, an association between serum IL-6 level and disease status has also been found in gastric carcinomas (17) and esophageal squamous cell carcinoma (16). Serum IL-6 levels in gastric carcinoma patients were found to increase in a stage-related manner and correlate with prognosis (17). The incidences of tumor invasion to adjacent organs and non-curative resection were significantly higher in esophageal carcinoma patients with elevated serum IL-6 levels (16). In addition, tissue concentrations of IL-6 in primary esophageal squamous cell tumors were ten times higher than those in normal epithelium (16). In addition, elevation of IL-6 has been shown to promote immune unresponsiveness and induction of wasting, cachexia and hypercalcemia, all of which are observed in patients with HNSCC who have a poor prognosis (16, 21). In breast cancer patients, significantly higher IL-6 levels were detected in patients with more than one metastatic site and with dominant metastatic visceral disease than in those with one metastatic site or with dominant metastatic bone or soft tissue disease (22). Patients with liver metastasis and pleural effusion have shown significantly higher serum IL-6 levels than those without either. Patients unresponsive to chemo-endocrine therapy have shown significantly higher serum IL-6 levels than those who did respond to such therapy (22). Moreover, patients with high IL-6 levels have shown significantly poorer survival than patients with low IL-6 levels. Multivariate analysis has revealed that IL-6, as well as disease-free interval, is an independent prognostic factor for metastatic breast cancer, suggesting that IL-6 levels are elevated in these patients and may be an aggressive parameter (22). In colorectal carcinoma, the serum IL-6 concentration in the peripheral venous blood has been strongly correlated with the concentration of IL-6 in the tumor tissue (6). In addition, the serum IL-6 concentration was significantly higher in patients with liver metastases and was correlated with tumor size. The authors concluded that the serum IL-6 level reflects the content of IL-6 in the

tumor component, and that the increase in the circulating serum level may reflect disease progress (6).

IL-6 exerts its action on target cells by acting through a receptor complex comprised of a specific IL-6-binding protein and a signal-transducing subunit (gp130). It has been reported that IL-6 and IL-6 receptor mRNAs were increased in colonic adenocarcinoma compared with normal colonic cells, and that certain characteristics associated with malignancy (such as cellular proliferation) could be regulated, in part, by locally produced IL-6 (23). The addition of a soluble form of IL-6 receptor together with IL-6 stimulated growth of Kaposi sarcoma cells that normally were not responsive to IL-6. Colorectal tumor tissues that expressed IL-6 immunoreactivity had a higher incidence of expression of IL-6 receptor immunoreactivity (6). Furthermore, a tendency for patients with both IL-6 and IL-6 receptor immunoreactivity to have higher serum IL-6 concentrations has been reported. These findings indicate that serum IL-6 levels may reflect the expression of both IL-6 and IL-6 receptor, thus suggesting the existence of an interaction between IL-6 and IL-6 receptor in the tumor (6).

It has also been suggested, from studies using cultured head and neck cancer cell lines and tumor specimens, that IL-6 expression may play a role in the increased pathogenicity of HNSCC by providing a growth advantage (24). This is consistent with the study by Dong *et al.* in which a murine model demonstrated a higher constitutive production of cytokine and resulted in a selective advantage of *in vivo* tumor growth and metastasis (25). Preliminary results of an analysis of the effect of surgery, chemotherapy or radiotherapy on IL-6 levels indicate that serum cytokine levels decrease in post-treatment patients (26).

In summary, our data on elevated serum levels of IL-6 in the majority of our HNSCC cancer patients suggest that serum IL-6 might be a candidate for a new marker for HNSCC, particularly for advanced stage tumors with metastatic potential to the lymph nodes. Serum IL-6 determinations may find clinical applications in the follow-up of cancer therapy. For this reason, studies with longitudinal follow-ups of patients are required to determine the value of serum IL-6 measurements in the detection of recurrent cancer.

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