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Serum Levels of IL-1β, IL-6, TNF-α, sTNF-RI and CRP in Patients with Oral Cavity Cancer

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Pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor- (TNF-α) play an essential role in the regulation of immune response to, and may have prognostic significance in, cancer. The aim of this study was to examine the relationship between the serum levels of IL-1β, IL-6 and TNF-α as well as the concentrations of soluble TNF receptor I (sTNF-RI) and C-reactive protein (CRP) in patients with squamous cell carcinoma of oral cavity. Results obtained were confronted with squamous cell carcinoma antigen (SCC) concentrations. IL-1β IL-6 and TNF-α serum levels as well as sTNF-RI and CRP concentrations were higher in patients than in controls. The increased serum levels appeared to be related to the clinical stage of disease. There was a correlation between IL-1β and sTNF-RI. IL-6 and IL-1β correlated with CRP levels. The mean concentrations of SCC were also elevated. IL-6 and sTNF-RI seemed to be the most sensitive parameters in early stages and may be used as additional markers in oral cancer (Pathology Oncology Research Vol 3, No 2, 126–129, 1997)

Key words: oral cancer, IL-1β, IL-6, TNF-α, sTNF-RI, CRP, SCC

Introduction

The clinical course of cancer results from interactions between tumor cells and the immune system of patients. Cytokines, such as IL-1β, IL-6 and TNF-α, play a key role in the regulation of the cellular and humoral immune responses to malignancies.[1-5] IL-1β, IL-6 and TNFα exhibit overlapping activities in various tissues and cells and their combinations could be synergistic or antagonistic.[4-8] IL-1β is similar in many of its actions to IL-6 and TNF-α. It was shown that both IL-1β and TNF-α are potent inducers of IL-6 production and activity. In contrast, IL-6 may inhibit IL-1β and TNF-α generation and showed an inhibitory effect on TNF-mediated monocyte cytotoxicity to tumor target cells.[4,6] IL-1β, IL-6 and TNF-α have an antiproliferative or cytocidal effect on several tumor cell lines, stimulating NK and macrophage-mediated tumor lysis, while augmenting the development of cytotoxic T lymphocytes and lymphokine activated killer (LAK) cells.[1,4-7]

Recently, much attention has been focused on cytokines role in the pathogenesis and progression of various malignancies. For example, IL-1β is a growth factor for acute and chronic myelogenous leukemia cells and thyroid carcinoma.[9] TNF-α has been reported to act as an autocrine growth factor in B cell cancer.[1] IL-6 is a potent growth factor for renal carcinoma, cervical carcinoma cell line, myeloma and others.[4] Since the release of cytokines by tumor cells has been also reported, the serum levels of these soluble mediators may be useful as additional cancer markers.

The aim of this study was to examine the relationship between the serum levels of IL-1β, IL-6 and TNF-α in patients with oral cavity carcinoma, confronted with clinical progression and concentration of SCC. We have also measured the levels of circulating TNF-regulatory protein, soluble TNF receptor I (sTNF-RI), and C-reactive protein (CRP).

Material and Methods

Fourty two patients (male and female) with squamous cell carcinoma of oral cavity were studied (*Table 1*). No infection or fever were present in any of the patients during

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Table 1. **Clinical characteristics of patients**

Sex	*N*	*Age (mean)*	*Tumor site*					*TNM classification*		
			mouth floor	*gingiva lower jaw*	*cheek*	*gingiva upper jaw*	*tongue*	*II*	*III*	*IV*
F	11	58.6	–	6	–	4	1	2	3	6
M	31	60.3	17	5	5	2	2	8	12	11

F – female, M – male, N – number of patients

examinations. Blood samples were taken from each patients at presentation before surgery. They did not receive chemotherapy before admission to the study. Obtained sera samples were stored frozen (-20°C to -70°C) until tested.

IL-1β assay – The concentration of IL-1β in the serum of patients was determined by ELISA – ENDOGEN Human IL-1β, according to the instructions. This ELISA kit is specific for measurement of natural and recombinant IL-1β. The concentrations of IL-1β for each sample was determined by reading values from the standard curve.

IL-6 assay – IL-6 was measured with ENDOGEN Human Interleukin-6 (IL-6) ELISA (Cambridge, USA), according to the instructions. The samples of serum were diluted 1:40. This ELISA is specific for measurement of natural and human recombinant IL-6. This kit has an assay range of 0-400 pg/ml of IL-6.

TNF-α assay – Samples of sera for presence of TNF-α were measured by ELISA using the Quantikine TNF-α Immuno assay from R&D System (Minneapolis, USA), according to the manufacturer's instructions. Recombinant TNF-α was used as a standard. This assay range is 15.6-1000 pg/ml TNF-α. The minimum detectable dose found by a standard curve was 4.4 pg/ml.

STNF-RI assay – Concentration of soluble TNF receptor was measured with ELISA – Quantikine sTNFRI, Immunoassay; R&D System (Minneapolis, USA), according to the manufacturer's instructions.

Each sample was determined in duplicate. The absorbance in all above mentioned assays was read at 450 nm using a Beckman spectrophotometer DU640.

CRP assay – CRP serum levels was measured with LC-Partigen Immunodiffusion Plates (Boehringwerke AG, Marburg).

SCC assay – Squamous cell carcinoma antigen (SCC) was determined by the IMx System obtained from Abbot. The assay is based on the Microparticle Enzyme Immunoassay typical sandwich-type protocol. A value of 1.5 ng/ml for SCC was considered as the upper limit of normal.

Statistical analysis – The results are expressed as mean±standard deviation. Data were analyzed according to variance and Student's t-test. Correlations were calculated using the Pearson's test. A p value less than 0.01 was cosidered to represent a statistically significant difference.

Results

Concentrations of IL-1β, IL-6 and TNF-α in the serum of patients were significantly different ($p<0.01$) from that of control subjects (*Table 2*). The most signficant differences between patients and controls were observed in the IL-6 serum level. The increased levels of IL-6 has been shown in 83%, IL-1β in 69%, and TNF-α in 52% of patients. Simultaneously elevated concentrations of IL-1β,

Table 2. **Mean serum concentrations of the mediators**

Factors	*Control* n=15	*Patients* n=42	*Stage II* n=10	*Stage III* n=19	*Stage IV* n=13
IL-1β (pg/ml)	30.5±4.68	123.3±93.2*	51.6±32.1**	149.7±112.8*[a]	190.2±104.9*
IL-6 (pg/ml)	10.3±5.07	79.6±42.1*	53.5±29.3*	81.2±51.2*	103.8±62.3*[b]
TNF-α (pg/ml)	12.7±4.89	45.8±37.01*	19.9±10.5	34.7±10.9*[a]	53.1±23.2*[b]
sTNF-RI (ng/ml)	2.97±0.87	6.52±1.61*	5.14±3.38*	6.23±3.26*	7.22±4.21*
CRP (mg/dl)	3.1±2.6	31.6±20.9*	7.66±5.71	16.81±12.9*	52.0±38.3*[b]
SCC (ng/ml)	1.27±0.32	1.92±0.77**	1.39±0.54	1.92±0.71	2.31±0.68*

* significant differences with control ($p<0.01$)
** significant differences with control ($p<0.05$)
a differences between patients in Stage II and III ($p<0.05$)
b differences between patients in Stage II and IV ($p<0.01$)

IL-6 and TNF-α were found in 41% of patients' serum with oral cancer. There was no correlation between these parameters in individual samples.

The serum level of sTNF-RI increased in 79% of patients, and the mean values of sTNF-RI concentrations were also significantly higher than those in controls ($p<0.01$) (*Table 2*).

The patients with oral cancer had significantly higher serum SCC and C-reactive protein levels than healthy controls. The increased serum level of CRP was found in 57% of cases.

The changes of all parameters were compared to the clinical stages estimated according to TNM classification (*Table 2*). It seems that tumor progression was accompanied by increased concentrations of these mediators. We have shown significantly higher IL-6 serum levels in patients in Stage II than in controls and for other parameters in patients in Stage III and IV than in controls. There was no significant difference in SCC levels between the different stages. In addition, there was a correlation between IL-6 and CRP concentrations ($r=0.63$, $p<0.01$), between IL-1β and CRP concentrations ($r=53$, $p<0.05$) and between the serum levels of sTNF-RI and IL-6 ($r=64$, $p<0.01$) in cancer patients. No correlation between SCC and other parameters was found. However, we did not observe any significant differences in examined parameters between the group of patients with various tumor sites and sex.

Discussion

Results of our study demonstrate markedly elevated serum levels of IL-1β, IL-6 and TNF-α as well as sTNF-RI, CRP and SCC in patients with oral cancer. The elevated serum levels of IL-1β, IL-6 and TNF-α have already been reported in patients with different solid tumors.[2,4,10-13] In patients with head and neck squamous cell carcinoma (HNSC), including oral carcinoma, increased serum level of IL-6 was also reported.[14]

Different mechanisms may be responsible for the high concentrations of IL-1β, IL-6 and TNF-α in oral cancer patients. IL-1β, IL-6 and TNF-α are produced by immunocompetent host cells such as monocyte/macrophage, T and B lymphocytes or polymorphonuclear cells.[3,6,9,15] The tumor cells may activate – directly or indirectly – these cells leading to increased cytokine production. Vitolo et al reported that peripheral blood lymphocytes (PBL) of patients with head and neck cancer produced IL-1β and TNF-α spontaneously after in vitro activation.[16] Tumor cells and intra- and peritumoral resident normal cells can also produce the cytokines and by doing that they modulate local and systemic immune response to tumors.[2,13,17]

The relatively small increase in the concentrations of TNF-α may be caused by the presence of circulating sTNF-RI. Soluble TNF receptor I is able to bind TNF-α without inducing signal transduction. It has been proposed that it can function as a natural inhibitor of TNF[11,18] Our results confirm the relevance of sTNF-RI concentrations in the serum of patients with cancer.[10] The soluble form of the RI (p60 or p55) receptor is approximately 100-fold more potent than sTNF-RII (p80 or p75) in inhibiting both the antiproliferative effects of TNF as well as in blocking TNF binding to respective cells.[9] Serum levels of sTNF RI and sTNF-RII were a more sensitive indicator of progressive cancer and had greater predictive value for detecting cancer than other markers, such as CA 125.[11,18]

Together with the action of other mediators, the regulatory effect of soluble TNF receptors on TNF-mediated reactions reflects probably their most important function.[11,18] The relationship between concentrations of IL-6 and sTNF-RI, observed in the present study, may be a key element in the homeostatic regulation of the pro-inflammatory cytokine network in cancer patients and appears to be a protective mechanism against the toxic effect of TNF-α.

Since IL-1β, IL-6 and TNF-α are known to stimulate hepatic protein synthesis, we examined this relationship between the concentrations of these cytokines and CRP levels.[5,6,7] The link, observed in oral cancers, is in accordance with other studies indicating a correlation between serum CRP and IL-6.[13] Moreover, we have also found the relationship between IL-1β and CRP. Increased serum levels of CRP in cancer patient may have several implications. CRP confers resistance against tumor growth in experimental animals and may form complexes with toxic cellular constituents such as result from tissue damage.[6,20] The high concentrations of IL-1β, IL-6 and CRP seems to confirm an important role of acute-phase response during malignant diseases, including oral carcinoma.

Cytokines controlling the acute phase response became a subject of extensive investigations. Some clinical and experimental studies indicate that the release of pro-inflammatory cytokines may be responsible for a high local recurrence rate after non radical surgery in patients with head-and-neck cancer.[21]

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