Serum Level of Interleukin-10 in Patients with Head and neck Squamous Cell Carcinoma

Mitra Farzin, Jannan Ghapanchi, Azadeh Andisheh Tadbir

Abstract: Objective: The objective of this study was to examine serum IL-10 levels in patients with HNSCC to elucidate its association with clinicopathologic parameters. Study design: Using an ELISA kit, we assessed the circulating levels of IL-10 in sera from 60 head and neck squamous cell carcinoma (HNSCC) patients as well as from 30 healthy controls. Results: The serum IL-10 concentration in HNSCC patients was significantly higher (14.08 ± 5.12 pg/ml, n=60) compared with healthy controls (4.4 ± 2.1 pg/ml, n=30; p<0.005). The serum levels of IL-10 were greater in patients with III/IV-stage tumors than in patients with I/II stage tumors. (19.3 ± 1.1 pg/ml, n=29 vs 9.1 ± 0.9 pg/ml, n=31, p<0.0005) and greater in T3/T4 tumor status than in T1/T2 tumor status (21.69 ± 2.41 pg/ml, n=19 vs 10.56 ± 1.31 pg/ml, n=41, p<0.0005). The serum concentration of IL-10 was greater in patients with nodal metastasis than in patients without nodal metastasis (19.1 ± 1.6 pg/ml, n=29 vs 9.4 ± 1.4 pg/ml, n=31). Conclusions: Serum IL-10 level is increased in HNSCC patients and this increase is an adverse prognostic factor.

Key words: IL10, Serum, Head and neck squamous cell carcinoma, ELISA

INTRODUCTION

Head and neck Squamous cell carcinoma (HNSCC) is an aggressive epithelial malignancy. It is the most common neoplasm arising in the upper aerodigestive tract and comprises a large group of tumors arising from the nasopharynx, oropharynx, hypopharynx and larynx. (Jebreel A, et al., 2007) HNSCC patients have been shown to exhibit profound immunosuppression (Fujieda S, et al., 1990). The mechanism by which tumor cells alter immunological function in the host is poorly understood. Therefore, research studies are focused on subclinical measurements of cell function, inflammatory mediators, cytokines and growth factors (Szaflarska A, et al., 2009). Cytokines are intercellular short-acting soluble mediators that are involved in the pathogenesis of cancer. (Guney N, et al., 2009)

and have been investigated in numerous studies as tumor markers, a prognostic tools for staging and survival and prognostic factors of post operative complications.

Interleukin (IL)-10 cytokine, is a homodimer with a molecular mass of 37 kDa. (Asadullah K, et al., 2003) Each monomer consists of 160 aminoacids with a molecular mass of 18.5 kDa (Asadullah K, et al., 2003). The human IL-10 gene is located on chromosome 1 and encodes for 5 exons (5.1 kb). This molecule was initially isolated by Mosmann et al (1986) and Fiorentino et al (1989).

IL10 is a pleiotropic cytokine produced by macrophages, T-helper-2 cells, and B Lymphocytes (Ikeguchi M, et al., 2009) and thought to play a potential or pathogenetic or therapeutic role in a number of human conditions, such as inflammation, autoimmunity and cancer (Howard M, O'Garra A. 1992).

A dual role has been proposed for IL10 in immunoregulation (Mocellin S, et al., 2003). Because many tumor types express IL10, its role in helping tumors evade immunosurveillance has been suggested (Young MR, et al., 1996). IL10 inhibits the tumoricidal capacity of macrophages and the cytotoxicity and cytokine production of tumor-specific T cells and blocks the presentation of tumor antigens by antigen-presenting cells (Rohrer JW, Coggin JH., 1995).

On the other hand in vivo studies in different animal models have demonstrated that IL10 is a potent inhibitor of tumor growth and metastasis (Beissert S, et al., 1995; Kundu N, et al., 1996).

Additionally, systemic administration of IL10 has inhibited tumor metastasis and stimulated antitumor immune responses in murine models (Berman RM, et al., 1996).

Over expression of IL10 is seen in many malignant disease including melanoma, basal cell and squamous carcinoma and renal cell carcinoma (Toiyama Y, et al., 2010).
Furthermore elevated serum levels of IL\textsubscript{10} have been identified in patients with melanoma, colon cancer, ovarian cancer, lung cancer, and lymphoma (Nemunaitis J, et al., 2001).

Data concerning the level of anti-inflammatory cytokine IL\textsubscript{10} in HNSCC patients display discrepancies. These discrepant observations prompted the present systemic studies on the occurrence and clinical significance of inflammatory (IL\textsubscript{10}) cytokine in the serum of patients with HNSCC.

**MATERIAL AND METHODS**

For the purposes of this study, 60 serum samples from patients diagnosed with HNSCC (38 males, 22 females, age: 64.3±12.4 years) and 30 serum samples from healthy control subjects (18 males, 12 females, age: 62.6±14.1 years) were collected. All the study patients were admitted to the ENT Department of Shiraz University of Medical Sciences and they had histopathological diagnosis of HNSCC. Patients with other maligncies were excluded. None of the patients had received chemotherapy or radiotherapy before surgery and did not show clinical symptoms of inflammatory disorders.

Control cases were healthy nonsmoker blood donors who had no evidence of systemic or inflammatory diseases, or infections, who were matched for age and sex.

The Ethical committee of the Shiraz University of Medical Sciences approved the study and informed consent was obtained from the all participants.

Serum sample were obtained from clotted blood following centrifugation at 4\degree C and stored at -80\degree C until analysis. IL\textsubscript{10} concentrations were measured by ELISA in accordance with the manufacturer’s instructions (BMS131/2, Bender MedSystems GmbH, Germany). The sensitivity of the ELISA test was (pg/ml).

Data of overall tumor stage, tumor T status, and nodal status were collected for each patients. The serum IL\textsubscript{10} concentrations were compared in patients by I/II-stage vs III/IV stage, T\textsubscript{1}/T\textsubscript{2} vs T\textsubscript{3}/T\textsubscript{4} as well as in patients with and without nodal involvement.

Independent t-test was performed to compare the results of serum IL\textsubscript{10} concentrations between controls and study participants. Mann-Whitney and Kruskal-Wallis tests were used to define the relation between serum IL\textsubscript{10} and clinical data. Differences were considered significant at p<0.05.

### Table 1: Characteristics of HNSCC patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
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<tr>
<td>Tumor site</td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td>51</td>
</tr>
<tr>
<td>Ororopharynx</td>
<td>5</td>
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<tr>
<td>Oral cavity</td>
<td>4</td>
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<tr>
<td>Tumor T status</td>
<td></td>
</tr>
<tr>
<td>T\textsubscript{1}</td>
<td>21</td>
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<tr>
<td>T\textsubscript{2}</td>
<td>20</td>
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<tr>
<td>T\textsubscript{3}</td>
<td>11</td>
</tr>
<tr>
<td>T\textsubscript{4}</td>
<td>8</td>
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<tr>
<td>Lymph node status</td>
<td></td>
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<tr>
<td>Node negative</td>
<td>31</td>
</tr>
<tr>
<td>Node Positive</td>
<td>29</td>
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<tr>
<td>Overall tumor stage</td>
<td></td>
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<tr>
<td>I</td>
<td>16</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
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<tr>
<td>III</td>
<td>14</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
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</tbody>
</table>

**Results:**

Table 1 shows the clinical data of patients assayed for serum IL\textsubscript{10}. There were 38 males and 22 females diagnosed with HNSCC in our study.

At the time of presentation, 31 patients (51.7%) were at stage I/II and 29 patients (48.3%) at stage III/IV. Regional lymph node involvement was present at 31 patients (51.7%) and all had localized tumor.

The serum IL\textsubscript{10} concentration in HNSCC patients was significantly higher (14.08 ± 5.12 pg/ml, n=60) compared with healthy controls (4.4±2.1 pg/ml, n=30; p<0.005).

There was no significant difference in IL\textsubscript{10} concentration between males and females, nor was there a correlation between serum IL\textsubscript{10} levels and age.

The serum levels of IL\textsubscript{10} were greater in patients with III/IV-stage tumors than in patients with I/II stage tumors. (19.3±1.12 pg/ml, n=29 vs 9.1±0.9 pg/ml, n=31, p<0.0005) and greater in T\textsubscript{1}/T\textsubscript{4} tumor status than in T\textsubscript{1}/T\textsubscript{2} tumor status (21.69±2.41 pg/ml, n=19 vs 10.56±1.31 pg/ml, n=41, p<0.0005)
The serum concentration of IL10 was greater in patients with nodal metastasis than in patients without nodal metastasis (19.1±1.6 pg/ml, n=29 vs 9.4± 1.4 pg/ml, n=31). (figure 1)

Fig. 1: IL-10 serum concentration in different groups of HNSCC patients.

Discussion:

In the present study, we demonstrated that serum IL-10 concentrations in HNSCC patients were statistically higher than control group and by using the different parameters such as primary tumor size, tumor stage and nodal status, this study showed that patients with advanced HNSCC, have significantly greater serum IL10 levels. Previous studies on circulating cytokines in HNSCC have reported discrepant observations.

Riedel et al. found that no IL-10 concentration elevation was seen in HNSCC patients than controls. (Riedel F, et al., 2004).

Hoffmann et al failed to detect IL 10 levels in the serum of patients with SCC and adenoid cystic carcinoma (Hoffmann TK, et al., 2007).

Alhamarneh showed serum IL10 detectability was significantly higher in HNSCC patients and pretreatment levels of IL10 in all anatomical subsides, except the oral cavity were significantly elevated in advanced diseases. However the detectable IL10 concentrations were not significantly different in levels between patients and controls. They found that IL10 detectability significantly correlated with poorer survival. (Alhamarneh O, et al., 2011)

In an intra-group comparison, Sparano et al revealed that patients with advanced HNSCC, had higher IL10 levels than patients with early stage disease (Sparano A, et al., 2004) and Lathers et al showed a significant increase in plasma levels of the Th2-type cytokines (Lathers DM, et al., 2003).

JeBreel et al stated that HNSCC patients were more likely to have detectable IL10 levels than were controls. (Jebreel A, et al., 2007)

The results of our study are in general agreement with those above, in that there was an increase in IL10 concentration in HNSCC patients and that advanced tumors have greater IL10 levels.

Several reasons are proposed for the relatively minor discrepancies observed in these studies including that studies used different media (plasma us serum) to detect IL10 levels, different tumor origins and different methods used to detect IL10 level.

Alhamarneh et al showed that the probability of having detectable serum IL10 levels was associated with the anatomical subsite. They revealed that tumors in the larynx, the oropharynx and the hypopharynx had a higher probability of having detectable IL10 levels than the oral cavity (Alhamarneh O, et al., 2011).

In the presents study, higher IL10 levels than other studies, might be due to that most of cases in this study, were laryngeal SCC.

A negative correlation between circulating levels of IL10 and prognosis has been reported in patients with solid tumors, including melanoma, lung cancer, colorectal, pancreatic, other advanced gastrointestinal carcinomas, and hematological malignancies including Hodgkin’s and non-Hodgkin’s lymphoma and leukemia’s (Bien E, et al., 2009).

In this study IL10 was most apparent in advanced disease stage, which is consistent with a suggestion that IL10 contributes to tumor progression.

IL10 has been strongly linked to immunoregulation and inflammation, and although the exact role is yet to be determined, several studies have shown IL10 to be a potent suppressor of the immune system through the inhibition of antigen-presenting cells, promoting of angiogenesis and down regulation of IL12 and IFN γ production (Alhamarneh O, et al., 2011).
It has also reported that IL-10 can block monocyte-dependent T-cell proliferation, induce energy in CD4+ T-cells and inhibit monocyte class II MHC expression (Nemunaitis J, et al., 2001). This local and possibly systemic effect of IL-10 may constitute a pathway for tumors to escape immune surveillance.

In summary, serum IL-10 level is increased in HNSCC patients and this increase is an adverse prognostic factor. So IL-10 production by HNSCC tumor cells may thus contribute to the tumor’s evasion of host immune response.

REFERENCES


