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Serum Interleukine-6 Concentration, But Not Interleukine-18, is Associated with Head and Neck Squamous Cell Carcinoma Progression

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Abstract Inflammation has been linked to various steps in tumorigenesis. Interleukin (IL)-6 and IL-18 are two inflammatory cytokines whose serum concentrations are elevated in several types of cancer, including head and neck squamous cell carcinoma (HNSCC) in some studies. This study was designed to analyze the serum concentrations of these cytokines in Iranian HNSCC patients. Serum IL-6 and IL-18 concentrations were assayed by ELISA commercial kits in 65 untreated patients and 20 healthy volunteers. Serum IL-6 concentration was significantly increased in patients compared to healthy individuals ($p<0.000$). IL-6 concentration increased as the tumor stage progressed, and a significant difference appeared between stage IV vs. stage I/II/III ($p=0.03$) disease. Although serum IL-18 concentration was higher in patients than in healthy individuals, the difference was not statistically significant ($p=0.06$). Moreover, there was no association between serum IL-18 concentration and tumor stage ($p=0.47$). A significant difference was observed in serum IL-18 concentration according to the gender with higher IL-18 concentration in male patients ($p=0.01$). In conclusion, serum concentration of IL-6 might correlate with the stage of tumor progression in Iranian HNSCC patients. Further studies with larger numbers of patients are required to exclude the possible minor correlation of serum IL-18 concentration with tumor stage.

Keywords Cytokine · Head and neck neoplasms · Interleukin-18 · Interleukin-6 · SCC

Abbreviations

ELISA	Enzyme-linked immunosorbent assay
HNSCC	Head and neck squamous cell carcinoma
IL	Interleukin
IFN-γ	Interferon-gamma
TNF-α	Tumor necrosis factor-alfa

Introduction

Head and neck squamous cell carcinomas (HNSCC), the sixth most common malignancy throughout the world, originate from the epithelial tissue of the upper aerodigestive tract including the oral cavity, pharynx, and larynx [1]. Despite improvements in diagnosis and conventional therapies, long-term survival rates in HNSCC patients have not increased significantly over the past three decades [1].

These carcinomas are characterized by a marked heterogeneity in their prognosis, and their pathogenesis is incompletely understood [1]. Inflammation has been linked to various steps in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [2]. Interleukin (IL)-6 and IL-18 are two inflammatory cytokines [3, 4] whose involvements in HNSCC have been suggested by several researchers [3, 5–10].

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IL-6, initially discovered as a B-cell growth factor, is a major inducer of acute phase of inflammation in combination with IL-1 and TNF-α [3]. This pleiotropic pro-inflammatory cytokine is produced by T- and B-cells, dendritic cells, macrophages, hematopietic cells, keratinocytes, fibroblasts, epithelial cells, astrocytes and several tumor cells [3, 11].

IL-6 might influence tumorigenesis through its effects on immune cells, inflammation, and cancer cells. It is a potent lymphoid growth factor with critical roles in the pathogenesis of lymphoproliferative malignant disorders [3]. IL-6 also augments chronic inflammation, and generates a microenvironment advantageous to cancer growth [2, 3]. Moreover, IL-6 is secreted by cancer cells such as HNSCC cells and participates in the regulation of cell proliferation and apoptosis [3, 5].

IL-18 is another pleiotropic pro-inflammatory cytokine that was first described as an IFN-γ inducing factor [4]. Like IL-6, it is also produced by many types of immune and non-immune cells including cancer cells [4].

IL-18 stimulates IFN-γ production synergistically with IL-12, promotes differentiation of T cells to a Th1 phenotype, and enhances the cytotoxic activities of NK cells and CD8+ lymphocytes [4]. It has been shown that IL-18 administration results in a significant suppression of tumor growth in animal models, suggesting a role for this cytokine in the host defense against cancer [4, 12]. However, IL-18 has the ability to stimulate IL-4 production in the absence of IL-12, endorse immune unresponsiveness, augment the adherence of cancer cells to microvascular walls, provoke the production of angiogenic factors and promote a prometastatic microenvironment [4, 12].

Serum concentrations of IL-6 and IL-18 were reported to be increased in a variety of inflammatory conditions ranging from autoimmune and infectious diseases [4] to malignancies, such as ovarian cancer [13], bladder cancer [14], esophageal cancer [15] and HNSCC [5–10]. The circulating concentrations of these cytokines were also correlated with cancer survival or clinical parameters in some studies [6, 7, 10, 12, 15], but not all [5, 13, 14, 16].

The present study investigated pretreatment serum concentrations of IL-6 and IL-18 in Iranian HNSCC patients and compared them to those in healthy volunteers. Also the possible correlation of serum concentrations of these two cytokines with sex, tumor location, and tumor stage at diagnosis was evaluated.

Materials and Methods

Subjects

The study was approved by the Ethics Committee of the Shiraz University of Medical Sciences. A total of 65 non-related patients (52 men and 13 women; mean age 59.4± 11.1 years) and 20 non-related healthy volunteers residing in the same region (16 men and 4 women; mean age 57.3± 10.2 years) were enrolled in this study. The patients were admitted to Khalili hospital in Shiraz, Iran. The diagnosis of squamous cell carcinoma was confirmed histopathologically. The stage of the disease was determined according to TNM classification.

Serum IL-6 and IL-18 Assays

Sera from 65 newly diagnosed patients and 20 age/sex-matched healthy volunteers were collected, aliquoted and stored at −70°C until use.

The serum concentrations of these cytokines were determined in duplicate by commercial quantities Enzyme linked Immunosorbent Assay (ELISA) kits (Biosource, USA) according to the instructions. The minimum detectable doses of the kits were <2 pg/ml and 12.5 pg/ml for IL-6 and IL-18, respectively

Statistical Analysis

The concentration of the IL-6 was not normally distributed in HNSCC patients according to the Kolmogorov-Smirnov test. Therefore, comparisons of IL-6 concentrations in different groups was performed using nonparametric tests (the Mann-Whitney U test or the Kruskal Wallis test). The concentration of the IL-18 was normally distributed, and Student's *t* test or one-way analysis of variance (ANOVA), as appropriate, was used to compare IL-18 concentration in different groups. The data were analyzed using SPSS software (version 11.5.0; SPSS, Chicago, IL, USA). Findings were considered statistically significant at a *P* value less than 0.05.

Results

The serum concentrations of IL-6 and IL-18 were checked in 65 untreated patients and 20 healthy control individuals. Table 1 indicates mean and median concentrations of IL-6 and IL-18 in patients and controls. The concentrations of both cytokines were higher in patients than in controls. These differences were significant in the case of IL-6 (*P* value<0.000) and nonsignificant in the case of IL-18 (*P* value = 0.06).

Serum IL-6 and IL-18 concentrations were analyzed according to the stage of the disease (Table 2). The circulating concentration of IL-6 increased as the stage of HNSCC progressed, and that, a significant increase appeared in stage IV disease compared to stage I/II/III

Table 1 Serum concentrations of IL-6 and IL-18 in HNSCC patients in comparison to healthy controls

Cytokine concentration	HNSCC patients (n=65)	Control group (n=20)	P value
IL-6 (pg/ml)			
Mean ± SD	6.6±7.4	undetectable[a]	<0.000[b]
Median (range)	3.8 (undetectable–38.0)	undetectable (undetectable-3.8)	
IL-18(pg/ml)			
Mean ± SD	473.5±277.3	347.1±224.3	0.06[c]
Median (range)	458.0 (32.0–1160.0)	303.0 (32.0–826.0)	

[a] undetectable: below limit of sensitivity of IL6 enzyme-immunoassay (<2 pg/ml)

[b] p value was calculated by the Mann-Whitney U test

[c] p value was calculated by Student's t test

disease (P value = 0.03). The circulating concentration of IL-18 was not different by the stage with a P value of 0.47.

The serum IL-18 concentration was significantly higher in male patients than female patients (p=0.01). Although the serum IL-6 concentration was increased in male patients compared with female patients, the increase was not statistically significant (p=0.08) (Table 2). In male and female control subjects, the serum IL-6 and IL-18 concentrations were similarly distributed (data not shown).

The tumor was located in the oral cavity in 23 cases (35.4%), in the pharynx in 8 cases (12.3%) and in the larynx in 34 cases (52.3%). Both IL-6 and IL-18 concentrations were not significantly different according to the tumor location.

Discussion

The association of HNSCC with inflammatory cytokines has been the subject of several studies [5–10, 16]. In the present study, the associations of serum IL-6 and IL-18 concentrations with HNSCC in Iranian patients were investigated. We found elevated circulating concentrations of these inflammatory cytokines in HNSCC patients compared to healthy controls that reached the statistical significance in the case of IL-6. The concentration of IL-6 was not normally distributed in HNSCC patients. This is similar to the study of Duffy et al. in which 444 HNSCC patients have been investigated [6].

It seems that the concentrations of these cytokines in cancer patients are elevated when compared to healthy control individuals in the majority of reports, but among the studies comparing cancer patients to cancer-free patients as control individuals, the evidence for a contribution is less apparent [3, 7, 8, 16]. For example, Riedel et al. determined the circulating concentration of IL-6 in sera from 90 German patients with HNSCC as well as from 39 healthy control individuals, and observed that the patients had significantly higher IL-6 compared to control group [7].

Table 2 Serum IL-6 and IL-18 concentrations according to gender, tumor localization, and tumor stage in 65 patients with HNSCC

Characteristics	n (%)	IL-6 concentration (pg/ml) Median (5th, 95th %)	P value[a]	IL-18 concentration (pg/ml) Mean ± SD	P value[b]
Sex			0.08		0.01
Male	52 (80.0)	4.3 (undetectable, 28.7)		515.1±277.7	
Female	13 (20.0)	3.0 (undetectable, 4.2)		307.3±212.1	
Tumor location			0.67		0.69
Oral cavity	23 (35.4)	3.4 (undetectable, 27.4)		475.3±304.6	
Pharynx	8 (12.3)	4.1 (undetectable, 38.0)		548.2±322.6	
Larynx	34 (52.3)	4.1 (undetectable, 20.1)		454.7±252.0	
Tumor stage[c]			0.03		0.47
I/II/III	46 (75.4)	3.3 (undetectable, 24.1)		485.8±297.5	
IV	15 (24.6)	4.8 (2.4, 38.0)		424.0±244.7	

[a] P values were calculated by Mann-Whitney U test or Kruskal Wallis test as appropriate

[b] P values were calculated by Student's t test or analysis of variance as appropriate

[c] The sum does not reach the total because of a few missing values

However, Jebreel et al. found no significant difference in serum IL-18 concentration between HNSCC patients and non-tumor control patients in UK [16].

Here, the serum concentration of IL-6 was increased by the progression of the tumor stage, and there was a significant elevation of IL-6 concentration in stage IV vs. stage I/II/III disease. Our results are in accordance with those of Duffy et al. [6], Riede et al. [7], and Jablonska et al. [10] in HNSCC patients. In contrast, Chen et al. [5] did not find such associations, which may be due to the limited numbers of their patients (only 11 cases).

Investigation of serum IL-18 concentration in 30 Polish patients with oral cancer and 15 healthy control individuals showed that the IL-18 concentration increased as the disease progressed [9]. This was not confirmed in the other study [16]. We also observed no association between serum IL-18 concentration and the stage of the disease. The discrepancy in results can partly be explained by the heterogeneity of disease [1], and the small sample size of the published study [9]. Despite the studies with large sample size on serum IL-6 in HNSCC [6, 7], so far, no study has been published to investigate the correlation between serum IL-18 concentration and HNSCC in a large number of patients.

Another finding in this study is the significant increase of IL-18 in male HNSSC patients compared to female HNSCC patients [6]. The other three serum IL-18 concentration studies in head and neck cancers did not investigate the association of HNSCC with the gender [8, 9, 16]. The higher concentration of serum IL-18 in men than in women was also observed in esophageal cancer (an organ near to the upper aerodigestive tract), but did not reach statistical significance (p=.08) [15].

It remains to be identified whether the increased serum levels of these cytokines is advantageous to cancer growth, or the host immune response especially in the case of IL-18, a cytokine with both pro-cancerous and anti-cancerous activities [12].

In conclusion, our date express that pretreatment serum concentration of IL-6, but not IL-18, is associated with HNSCC and progression in tumor stage, a key prognostic factor in HNSCC [1]. More data comprising a larger numbers of patients are required to exclude the possible minor association of serum IL-18 concentration with HNSCC.

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