RESEARCH ARTICLE

Editorial Process: Submission:05/09/2017 Acceptance:01/12/2018

Serum CXCL12, but not CXCR4, Is Associated with Head and Neck Squamous Cell Carcinomas

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Abstract

Background: Squamous cell carcinoma (SCC) is the most frequent malignancy of the head and neck (HN) region. We here evaluated associations of stromal cell derived factor-1 (SDF-1or CXCL12) and its receptor, CXCR4, with HNSCCs. **Materials and Methods:** Sixty newly diagnosed HNSCC patients were enrolled in the patient group, and 28 healthy individuals in the control group. Plasma levels of CXCL12 and CXCR4 were measured using ELISA kits. **Results:** There was a significant difference in mean CXCL12, but not CXCR4, plasma levels between the patient and control groups (P=0.0001). No significant associations were found between mean plasma levels of either CXCL12 or CXCR4 with age, gender, tumor site, tumor size, lymph-node involvement or tumor stage. **Conclusion:** For the first time, our findings demonstrate a significant association between serum CXCL12 but not CXCR4 levels and HNSCCs.

Keywords: CXCL12- CXCR4- HNSCC- serum

Asian Pac J Cancer Prev, **19 (4)**, 901-904

Introduction

Squamous cell carcinoma is the most frequent malignancy of the head and neck region. Head and neck squamous cell carcinoma (HNSCC) develops from the mucosal linings of some tissues and organs around head and neck. The most common sites are hypopharynx, larynx, trachea, oral cavity and oropharynx (Ghapanchi et al.,2014). The incidence of HNSCC is rising all over the world (Mousavi et al., 2009), and identification of new easily-obtained biomarkers is of particular interest.

Chemokines are a family of cytokines regulating chemotaxis (Lavaee et al., 2018). Among them, stromal cell derived factor-1 (SDF-1; also called as CXCL12) and its receptor, CXCR4, play an important role in the migration of cells, including cancer cells and immune cells. The signaling pathway consisting of CXCL12 and CXCR4 is closely correlated to growth, invasion and metastasis of several types of cancers, such as (Muller et al., 2001), kidney (Schrader et al., 2002), ovary (Scotton et al., 2001), prostate (Taichman et al., 2002), brain (Zhou et al., 2002), lung (Kijima et al., 2002), thyroid (Hwang et al., 2003) and HNSCC (Almofti et al., 2004).

Most of investigations on CXCL12/CXCR4 expression are conducted on tissues or cells rather than body fluids. For example, Almofti et al., (2004) studied oral SCC and expression of CXCL12and CXCR4 in biopsy specimens by immunohistochemistry. They did not find a statistically significant association between the expression of CXCL12 and any clinicopathological parameter. However, they found a significant association between the expression of CXCR4 and lymph node metastasis, the mode of invasion and recurrence of the tumors. Taichman et al., (2002) investigated the role of CXCR4/CXCL12 in prostate cancer spread to bone. By reverse transcription-PCR and Western blotting, they showed that levels of CXCR4 expression were higher in several human prostate cancer cell lines derived from malignancies metastasized to bone. Prostate cancer cells, in response to SDF-1, also showed an increase in migration across bone marrow endothelial cell monolayers, and invasion through basement membranes. Hwang et al., (2003) conducted some research on CXCR4 in human anaplastic thyroid cancer cells. They mentioned that a subset of anaplastic thyroid carcinoma cells expresses functional CXCR4 which may be important in tumor cells migration and invasion.

Identification of a biomarker in serum has advantage over tissue because the specimen can be obtained non-invasively, and also inexpensive at sufficient amount. We have previously studied pretreatment systemic levels of IL-6, IL-7, IL-8, IL-4, IL-10, and IL-18 in HNSCC. Consistent with other publications, we found systemic levels of IL-4, IL-6, and IL-7 as a possible biomarker in HNSCC (Mojtahedi et al., 2011; Mojtahedi et al., 2012; Mojtahedi et al., 2014). Systemic levels of CXCR4 and CXCL12 have been recently investigated in several types of cancer (Lim and Chung 2015; Choi et al., 2016), but

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not HNSCC.

In the present study, for the first time, we aimed at evaluating systemic levels of CXCR4 and CXCL12 in HNSCC compared to healthy individuals. We further evaluated the associations between systemic levels of these two molecules and other clinicopathological characteristics of the patients at diagnosis.

Materials and Methods

The present case-control study, which was conducted during 2015-2016, was approved by the local Ethics Committee of our university. All participants were informed that blood samples would be used in research projects, and their consent was obtained.

The patients had been referred to the Oral and Maxillofacial Disease and ENT Department of Dental Faculty. HNSCC was confirmed by biopsy and pathologist assessment. Patients did not have any other systemic diseases interfering with HNSCC, including any other cancer, autoimmune, inflammatory or infectious diseases. In total, 60 patients with newly diagnosed HNSCC were enrolled in the patient group. Patient characteristics including gender, age, smoking status, location of tumor, grade and stage of SCC at the time of diagnosis were obtained from their files. Tumor-node-metastasis (TNM) classification system was used for determining the stage of disease. In the control group, 28 non-related healthy individuals who referred to the Oral and Maxillofacial Disease Department for their Dental problems were enrolled. They were healthy with no history of malignant, metabolic, inflammatory or autoimmune diseases.

Each participant was examined for any sign of infection. Cell blood count was requested for all of them. No evidence of acute infection in a past month was found in participants. Blood samples were taken from peripheral venous blood of patients and controls. Plasma was collected in 2 hours from sampling, and stored at -70°C until use.

The plasma levels of CXCL12 and CXCR4 were measured in duplicate using commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, USA) according to the protocols of manufacture. The detectable limits of the kits were 1.0-47 pg/ml and 15.6-1,000 pg/ml for CXCL12 and CXCR4, respectively. Concentrations below the detection limits were considered zero in statistical analyses in both patient and control groups.

Analysis of variance (ANOVA) and t-test were used to calculate differences in the levels of CXCL12 and CXCR4 between patients and controls, and to assess their associations with clinical characteristics of patients. Data were analyzed using the Statistical Package for the Social Sciences version 11.5.0 software program (SPSS Inc, Chicago, Illinois, USA). A P value of less than 0.05 was considered statistically significant.

Results

In this study, the plasma levels of CXCL12 and CXCR4 were measured in HNSCC patients and a healthy group. We investigated 60 HNSCC patients, including

Table 1. Plasma Levels of CXCR4 and CXCL12 in Head and Neck Squamous Cell Carcinoma Patients and Healthy Controls

Variable (pg/ml)	Patients	Controls	P-values
CXCR4 (Mean± SD)	640.6±111.1	684.1±123.7	0.111
CCL12 (Mean± SD)	3993.8±623.5	2,971.9±740.5	0.0001

Table 2. Mean Plasma Levels of CXCR4 and CXCL12 According to Gender, Tumor Site (Larynx, Tongue, Oral Cavity), and Tumor Size

Parameter	N (%)	CXCR4 (pg/ml) Mean±SD	CXCL12 (pg/ml) Mean±SD	P-values
Male patients	42 (70)	650.5±110.5	3,966.6±607.1	0.298*
Female patients	18 (30)	617.6±112.3	4,057.4±674.0	0.609**
Male controls	19 (68)	689.01±134.6	2,793.8±577.0	0.791*
Female controls	9 (22)	675.0±107.1	3,347.8±931.4	0.063**
Tumor site				
Tongue	21 (35)	627.1±110.6	3,992.2±637.2	0.737*
Larynx	32 (53.4)	651.1±112.0	3,939.1±614.3	0.499**
Oral cavity	7 (11.6)	633.2±120.2	4,249.1±654.0	
Total	60 (100)	640.6±111.1	3,993.8±623.5	
Tumor size				
≤2 cm	10 (16.6)	654.7±95.9	4,108.6±543.6	0.701*
2-4 cm	29 (48.4)	631.5±109.1	3,992.0±587.0	0.920**
≥4 cm or invades adjacent tissues	21 (35)	646.5±123.9	3,941.8±722.6	
Total	60 (100)	640.6±111.1	3,993.8±623.5	
Lymph node involvement				
0	41(68.4)	639.8±115.1	4,038.1±607.3	0.932*
1 or more	19 (31.6)	642.4±104.9	3,898.3±663.8	0.424**

*P-values related to CXCR4 levels; ** P-values related to CXCL12 levels.

42 men (70.0%) and 18 (30.0%) women, and 28 healthy individuals consisted of 19 (67.9%) men and 9 (32.1%) women. Patients were non-related with mean age of 62.68 ± 14.49 years. The controls were also non-related with similar mean age as the patients.

The mean plasma levels of CXCL12 and CXCR4 in patient and control groups are presented in Table 1. As indicated the difference in the mean plasma levels of CXCR4 in case and control groups is not statistically significant (P=0.15). However, the CXCL12 plasma levels were higher in patients compared to the control group and the difference was highly significant (P= 0.0001).

The mean plasma levels of CXCR4 and CXCL12in patient and control groups by gender, tumor site, tumor size, and lymph node involvement are presented in Table 2. They were not associated with mean plasma levels of the two measured molecules Table 2.

Mean plasma levels of CXC4 and CXCL12 were also analyzed according to tumor stage. There was no significant association between plasma levels (pg/ml) and tumor stage (mean plasma levels± SD of CXCR4 in stage I/II and III/IV were 657.7± 117.4, and 623.5± 103.5, respectively with P value 0.29; mean plasma levels± SD of CXCL12 in stage I/II and III/IV were 4083.7± 565.5, and 3904.0± 674.1, respectively with P value 0.30).

Metastasis was confirmed in one patient at diagnosis, and thus it was not possible to analyze the relationship of metastasis and serum levels of CXCL12 and CXCR4.

Discussion

In our study, there were statistical significant differences between mean plasma levels of CXCL12 in patient and control groups. On the other hand, there were no significant differences in mean plasma levels of CXCR4 between patient and control groups. Moreover, there were no significant associations between mean plasma levels of CXCL12 and CXCR4 and age, tumor site, tumor size, lymph node involvement, and tumor stage.

Investigations of tissues/cells regarding local expression of CXCL12 and CXCR4 were repeatedly reported positive, particularly for CXCR4. Using immunohistochemistry, Tan et al., (2008) have found the expressions of CXCR4 (53.5% of samples were positive) and CXCL12 (40% of samples were positive) were significantly higher in HNSCC tumor tissues compared to adjacent non-tumor tissues. Greater expression of CXCR4, but not that of CXCL12, was associated with lymph node involvement and distant metastasis. CXCR4 proteins were detected in the cytoplasm and/or cell membranes of cancer cells, but not in the normal stromal cells of adjacent non-tumor tissues. Negative or weak CXCR4 protein staining was found in the most of infiltrating inflammatory cells. On the other hand, CXCL12 protein was highly expressed not only in the cytoplasm of stromal cells and lymphocytes adjacent to cancer cells but also in the cytoplasm of some cancer cells (Tan et al., 2008). In the study of Almofti et al. (2004), the expressions of CXCR4 and CXCL12 were reported in 57.3 and 11.4% of HNSCC cases, respectively. No statistically significant association between the expression of CXCL12 and any clinicopathological factors were found, while a significant association between the expression of CXCR4 and lymph node metastasis, the mode of invasion, and recurrence of HNSCC was found. Albert et al., (2013) reported expressions of CXCR4 and CXCL12 in 87%, and 95% of tumors, respectively, compared to normal epithelial cells adjacent to the tumor tissues. Regarding with clinicopathological characteristics of the patients, they observed a significant increase in expression of CXCR4 in patients with high-grade tumors, lymph node metastasis, and microscopic nerve invasion and poor overall survival. On the other hand, no significant correlation was found between CXCL12 expression and pathologic stage of tumor. Clatot et al., (2011) and Uchida et al., (2003), in contrast to the above studies (Almofti et al., 2004; Katayama et al., 2005; Lee et al., 2009), did not detect any correlation between CXCR4 expression in HNSCC and its prognosis. Different methods of evaluation (IHC vs. PT-PCR) and specificity of CXCR4 antibodies can justify the contradictory findings.

Contrary to studies published on local tissue expression of CXCR4/CXCL12, which showed a relation between these two molecules, particularly CXCR4, with characteristics of the patients, the results of our investigation on systemic body fluids, displayed a different trend. In our study, CXCL12, but not CXCR4, significantly had higher plasma levels in comparison with healthy controls. Also, we did not observe a strong correlation between CXCR4/CXCL12 systemic levels and indication of tumor aggressiveness. The differences in our results with those published on HNSCC tissues can be explained by different sources of specimen in these studies (local versus systemic levels). CXCL12 is a secretory ligand for CXCR4 and may be released in body fluids, and its expression levels in the tissue samples might be less predictable, especially in comparison with CXCR4, which is a tissue receptor, and its levels in tissue may be a more accurate biomarker in HNSCC.

To the best of our knowledge, there is no report on systemic levels of CXCL12 and CXCR4 in HNSCC. However, there are a number of publications regarding the systemic levels of these two chemokines and other types of cancer. Vizio et al., (2010) investigated the plasma levels of CXCL12 in pancreatic cancer patient. They found significant higher expression of CXCL12 neither in pancreatic cancer patients compared to healthy controls nor any associations between CXCL12 plasma levels and characteristics of the patients. Lim et al., (2015) investigated CXCL12 in gastric cancer patients. They did not include a healthy control in their study. They found that serum CXCL12 could predict distant metastasis in gastric cancer. In colorectal cancer, Choi et al., (2016) found that serum CXCR4 level was positively correlated with metastatic sites, and liver metastasis. They did not compare their data with a healthy control group.

Our study had some limitations. We did not have any information about HPV infection and different habits, and the patient environment of our studied groups. Increasing the number of our studied groups could eliminate any effects of confounding factors. Simultaneous evaluations

of tissue expression of CXCL12 and CXCR4 with their plasma levels could also shed more light on comparison of the values of tissue vs. plasma of these two molecules as a biomarker in HNSCC.

Our results demonstrate a significant association between systemic levels of CXCL12 and HNSCC but no significant association between systemic levels of CXCR4 and HNSCC. Systemic levels of CXCL12 as a HNSCC biomarker are recommended to be evaluated in other populations. Future studies will reveal whether the increase in CXCL12 levels is the cause or consequence of HNSCC.

Acknowledgments

The authors thank the Vice-Chancellery of Shiraz University of Medical Science for supporting the accomplishment of this research (Grant# 8894104). This manuscript is based on the thesis done by Dr. Sahar Zare. The authors also thank Dr. Mehrdad Vosoughi of the Center for Research Improvement of the School of Dentistry for the statistical analysis.

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