RESEARCH ARTICLE

Decreased Serum Monocyte Chemoattractant Protein-1 in Salivary Gland Tumor Patients

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Abstract

Background: The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a potent chemoattractant for natural killer cells, monocytes, and memory T lymphocytes. However, any role in the genesis of salivary gland tumors (SGT) is unknown. To assess the diagnostic relevance of chemokines in SGT, MCP-1 levels in the serum of patients were investigated in association with tumor progression and clinical aggressiveness. Materials and Methods: Using an ELISA kit, we assessed and compared the circulating levels of MCP-1 in blood serum of 70 SGT patients with 44 healthy control samples. Results: The results of this study showed that the concentration of MCP-1 was significantly lower in patients with benign (463.8±158.5pg/ml, P=0.033) and malignant (454.8±190.4pg/ml, P=0.007) SGT than in healthy subjects (645.7±338.9). No significant difference in mean serum levels of MCP-1 was observed between the benign and malignant group (p=0.9). While MCP-1 levels were lower in patients with an advanced clinical stage, advanced tumor size, higher tumor grade, or lymph node involvement, but the mean MCP-1 level between groups showed no statistically significant difference (p>0.05). Conclusions: MCP-1 levels in the serum of patients with SGT were decreased, indicating that this might a good marker for discriminating patients with SGT from healthy people. However, no clear-cut relationship was detected between MCP-1 levels and clinicopathologic factors, and MCP-1 is not a good marker for evaluating tumor dissemination.

Keywords: Salivary gland tumor - MCP-1 - pleomorphic adenoma - adenoid cystic carcinoma - mucoepidermoid

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Introduction

Chemokines form a kind of low-molecular-weight, inducible, proinflammatory chemotactic cytokine which is involved in a variety of biological processes, such as angiogenesis, the migration of leukocytes, and tumor growth (Wu et al., 2013; Sambyal et al., 2015). Chemokines are divided into four groups (CXC, CC, C, and the CX3C family)according to the organization of positionally conserved cysteine residues (Heywood et al., 1998).

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the CC subgroup of chemokines and firstly was cloned from human myelomonocytic and glioma cells(Matsushima et al., 1989). MCP-1 is a 76-amino acid protein, 13 KDa in size, that is located on chromosome 17 (Deshmane et al., 2009). It is a potent chemoattractant for natural killer cells, monocytes, and memory T lymphocytes (Bolat et al., 2006). MCP-1 production can be seen in many different cells such as astrocytes, mast cells, fibroblasts, monocytes, and endothelial cells in response to inflammation (Ishioka et al., 2013). It also can be produced by human and murine malignant cells (Yoshimura et al., 1989; Deshmane et al., 2009). Several studies have reported that serum MCP-1 expression is associated with tumor progression, lymph node metastasis, stroma formation, and clinical aggressiveness (Nakashima et al., 1998; Ueno et al., 2000; Loberg et al., 2007). Salivary gland tumor (SGT) accounting for 3-10% of all head and neck tumors (Jones et al., 2008;Ashkavani et al., 2013). The role of MCP-1in the tumorogenesis of SGT is unknown. To assess the diagnostic relevance of chemokine in SGT, MCP-1 levels in the serum of patients with SGT were investigated and tested for associations with clinical and pathological parameters.

Materials and Methods

In this case-control study, serum samples from 70 patients diagnosed with SGT (30 males, 40 females, age: 44.8±16.6 years), including 44 cases of pleomorphic adenoma, 7 of adenoid cystic carcinoma, and 19 of mucoepidermoid carcinoma, and 44 serum samples

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from healthy control subjects (19 males, 25 females; age=44.6±16.6 years) were collected. Study subjects were patients admitted to the Department of Otolaryngology, Khalili Hospital, Shiraz University of Medical Sciences and histopathologically diagnosed with SGT. Control cases were healthy blood donors who were matched to patients for age and gender. Exclusion criteria for both groups included the presence of any systemic disease,use of corticosteroid or non steroid anti-inflammatory medication,or a history of malignancy of any type. All participants were informed about the study and agreed to participate by signing an informed consent form. The Ethical Committee of the Shiraz University of Medical Sciences approved the study.

Serum samples were obtained from clotted blood following centrifugation at 4°C and were stored at -80°C until analysis. MCP-1 concentrations were measured by ELISA in accordance with the manufacturer's instructions (BMS281, Bender Med Systems GmbH, Germany). Anova and Tukey tests were performed to compare serum MCP-1 concentrations between controls and study participants. T test and Spearman's correlation test were used to define the relationships between serum MCP-1 and clinical data. Differences were considered significant at $p<0.05$.

Results

The results of this study showed that the concentration of MCP-1 was significantly lower in patients with benign (463.8±158.5pg/ml, P=0.033) and malignant (454.8±190.4pg/ml, P=0.007) SGT than in healthy subjects(645.7±338.9). No significant difference in serum levels of MCP-1 was observed between the benign and malignant group (p=0.9).There was no significant difference in MCP-1 concentration between males and females (p=0.2); nor was there a correlation between MCP-1 levels and age (P=0.7). MCP-1 levels were lower in patients with an advanced clinical stage, advanced tumor size, higher tumor grade, or lymph node involvement, but the mean MCP-1 level between groups showed no statistically significant difference ($p>0.05$) (Table1).

An ROC analysis indicated that MCP-1 was a diagnostic biomarker useful in discriminating patients with SGT from healthy people (AUC=0.68, CI: 95%). The optimal value of MCP-1 for discriminating patients from healthy subjects is 450.5 pg/ml.

Table 1. Clinicopathological Features and MCP-1 Levels of the Patients with Malignant Salivary Gland Tumors Included in this Study

	Number (%)	Mean MCP-1 Level ± SD	P-value
Sex			
Male	9 (34.7)	467.2 ± 241.2	0. 8
Female	17 (65.3)	430.7 ± 154.2	
Tumor size			
T1 + T2	18 (69.2)	508.4 ± 247.2	0.3
T3 + T4	8 (30.8)	435.8 ± 165.6	
Lymph node involvement			
N0	20 (76.9)	508.1 ± 207.2	0.8
N1	6 (23.1)	441.6 ± 217.3	
Distant Metastasis			
M0	23 (88.4)	478.8 ± 212.8	0.7
M1	3 (11.6)	457.3 ± 196.4	
Grade			
G1	8 (30.7)	498.5 ± 105.2	
G2	10 (38.6)	452.3 ± 261.2	0.2
G3	8 (30.7)	410.1 ± 167.2	
Stage			
I + II	15 (57.6)	505.3 ± 189.4	0.4
III + IV	11 (42.4)	422.7 ± 195.3	

Discussion

Chemokines are small, secreted chemotactic cytokines that coordinate immunological machinery by promoting leukocyte migration and cross-communication among different cell types of the immune system (Waugh et al., 2008). Due to similarities between leukocyte trafficking and cancer cell dissemination, the role of chemokines in tumorogenesis is increasingly drawing attention (Koizumi et al., 2007).

Recent studies regarding various tumor entities point to the significant participation of chemokines in tumor initiation and progression (Tsaur et al. 2011). For the first time, this study explored the potential of MCP-1chemokines as biomarkers in patients with salivary gland tumor (SGT).MCP-1/CCL2 is the most representative member of the CC chemokine superfamily that plays an important role in the recruitment of monocytes, lymphocytes, and dendritic cells.MCP-1 is mainly expressed by stromal cells, e.g., endothelial and inflammatory cells, and its upregulation is seen in response to proinflammatory stimuli and after tissue injury. Its role in different cancers, however, is still controversial. In this study, MCP-1 was shown to be significantly lower in the serums of benign and malignant SGT patients when compared with those of healthy controls. These results demonstrated that decreased MCP-1concentration is associated with the initiation of SGT.

Mono-nuclear cells are considered to be main contributors to restricting cancer growth (Klintrup et al. 2005), and clinical studies have indicated that the level of tumor-derived MCP-1 is significantly correlated with the density of mono-nuclear cells in different tumors (Negus et al., 1995; Ohta et al., 2003; Valkovic et al., 1998). It is concluded that lower levels of MCP-1 result in smaller percentages of infiltration into T cell, macrophages, and natural killer cells and increased tumor progression.

Several studies have investigated the circulating levels of MCP-1 in different malignant tumors. Elevated serum levels of MCP-1 were observed in patients with pancreatic and gastric cancers, nasopharyngeal and hepacellular carcinomas (Wang et al., 2013; Andisheh-Tadbir et al., 2014). Conversely, decreased levels of serum MCP-1 were reported in cases of colorectal and gastric cancers and oral squamous cell carcinoma (Deshmane et al., 2009; Andisheh-Tadbir et al., 2014; Ding et al., 2014; Kantola et al., 2012).The exact mechanism resulting in this contradiction is not clear. Tonouchi et al. assumed that decreased MCP-1 levels may reflect increased local

consumption in tumor (Tonouchi et al., 2002). Previous studies have shown the utility of MCP-1 for differentiating between benign and malignant lesions in ovarian cancer and gliomas (Church, et al., 2005; Christiansen et al., 2005). In the present study, no significant difference was seen between benign and malignant SGTs.

The present study found that patients with advanced tumor (T, N, and M) have lower MCP-1 levels compared with patients with early SGT, but the difference was not statistically significant. Low serum levels of MCP-1 in an advanced stage confirms the inadequate recruitment of mononuclear inflammatory cells. The recruitment of mononuclear cells is necessary for an effective anti-tumor cytotoxic response. A study on hepatocellular carcinoma indicated that the level of MCP-1 is related to disease stage (Wang et al., 2013). Tonouchi et al. reported a decreased level of MCP-1 in accordance with disease progression in gastric cancer patients (Tonouchi et al., 2002). The lack of associations between MCP-1 and metastasis, lymph node involvement, and histological grade of pancreatic tumor suggested that this chemokine is not relevant to tumor dissemination (Monti et al., 2003). Thus, a dual role for MCP-1 on tumor growth was proposed (Huang et al., 1994; Nakashima et al., 1995).

Nesbit et al. showed that lower MCP-1 concentration caused tumor formation via physiologic TAM (tumor-associated macrophages) accumulation, while higher MCP-1 level result in maximum macrophage infiltration and subsequent tumor destruction (Nesbit et al., 2001). Thus the definite roles of tumor-infiltrating leukocytes and chemokines in tumor progression may be different depending on tumor type. This result points out that the biological implication of chemokines in the tumor microenvironment is particularly complex and has not been entirely clarified.

As the findings of this study are limited to surgically resected SGT patients, the temporal relationship between MCP-1 level changes in serum and the progression of SGT remains unknown. Therefore, additional studies on this protein in unresectable malignant lesions is recommended.

In conclusion, MCP-1 levels in the serum of patients with SGT were decreased, indicating that its serum level is a good diagnostic marker for discriminating patients with SGT from healthy people. However, no clear-cut relationship was detected between MCP-1 levels and clinicopathologic factors, and MCP-1 is not a good marker for evaluating tumor dissemination.

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