RESEARCH ARTICLE

Serum Level of Mast Cell Tryptase in Patients with Oral Squamous Cell Carcinoma: Lack of Correlation with Clinicopathologic Factors

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Abstract

Background: Mast cells can influence tumor progression via different pathways and increased mast cell density has been demonstrated in oral squamous cell carcinoma (OSCC). It has been shown that the serum tryptase level is elevated with some malignant tumours and may thus be a useful parameter. However, there are no data available about OSCC. The main aim of this study was the evaluation of mast cell tryptase (MCT) level in OSCC patient serum. Materials and Methods: In this cross-sectional, analytic study, the circulating levels of MCT were assessed in sera of 55 OSCC patients and 34 healthy individuals with ELISA technique. Results: The serum MCT level in OSCC patients was 12-14 ng/ml, which was not significantly higher than the healthy control group. While the serum level of MCT was higher with larger tumours, there was no apparent correlation with clinico-pathological features such as patient age, gender, tumor location, stage, nodal status, distant metastasis, histological grade and smoking. Conclusions: Our findings showed that despite the results obtained from studies of other malignant tumors, serum level of MCT in OSCC patients could not be a credited as a reliable indicator of the presence or progression of tumours.

Keywords: Mast cell - tryptase - serum - oral - head and neck - squamous cell carcinoma

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Introduction

Squamous cell carcinoma (SCC) is the most common malignancy in the oral cavity. Despite the increasing knowledge and developing diagnostic and therapeutic methods, the survival rate of patients is low (Messadi et al., 2009). Recently, the molecular and biological factors that may be helpful in early diagnosis and treatment of the patients, have broadly studied. However, up to now there are not any universally accepted diagnostic and prognostic markers. In this regard, much attention has been given to the tumor environment, frequently. Inflammatory cells are present in the tumor stroma and can affect tumor development and growth (Ribatti and Crenellate, 2009). Among these immune cells, the roles of mast cells have been demonstrated in various biologic processes in the many tumors (Artuc et al., 2002; Fukushima et al., 2006; Ribatti and Crivellato, 2009). Mast cells are heavily granulated cells which are derived from bone marrow and migrate to the other organs, where they exert their functions (Galli et al., 2005; Sayed et al., 2008). The major role of mast cells is releasing some bioactive materials. Mast cells play some roles in tumor progression via increasing angiogenesis (Norrby, 2002). Some proteases such as tryptase and chymase are stored in the mast cell granules (Ribatti and Crivellato, 2009).

These proteases can degrade the extracellular matrix and promote tumor invasion and metastasis. Mast cells accumulate around many malignant tumors such as cutaneous malignancies, breast cancer and melanoma (Ribatti et al., 2003; Ch'ng et al., 2006; Rajput et al., 2007; Xiang et al., 2010). Also, increased mast cell density has been shown in oral and esophageal SCC (Iamaroon et al., 2003; Elpek and Gelen, 2005; Rojas et al., 2005; Jaafari-Ashkavandi et al., 2010; Tinge et al., 2010;). The positive correlation has been shown between mast cell density and poor prognosis of oral SCC (OSCC) (Iamaroon et al., 2003), even though there are controversies around this issue (Oliveira-Neto et al., 2007; Cheema et al., 2012). According to these results, some authors have considered mast cells as a target for cancer therapy (Nechushtan, 2009). Other researchers assessed the possible clinical relevance of mast cell degranulation in some malignant tumors (Samoszuk and Corwin, 2003).

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There are a few precise, available laboratory methods to determine mast cell activation: histamine and its metabolites in plasma or urine, also the serum level of mast cell tryptase (MCT) (Valent et al., 2011). MCT level is an indicator of mast cell quantity and activation (Hogan and Schwartz, 1997). There are two main types of MCT: α and β-tryptase α-tryptase is secreted spontaneously as inactive proenzyme and is found more than β-tryptase in blood of normal subjects. β- Tryptase is released during degranulation in proteolytic processes, such as inflammation and in poor vascular areas (Valent et al., 2011).

Increased MCT in level in sera of patients with breast cancer has been shown, previously (Samoszuk and Corwin, 2003), One study showed their importance as a diagnostic and prognostic marker for distinguishing pancreatic cancer from benign tumors in the same location (Strouch et al., 2010). According to the previous reports; we hypothesized that mast cell activation could be a useful parameter for evaluating OSCC patients.

The aim of this study was to determine the serum level of MCT in OSCC patients and analysis the correlation of this level with clinicopathologic parameters.

Materials and Methods

Patients

In this cross-sectional, analytical study, we used blood samples from 55 patients with OSCC whom referred to the ENT department of Khalili hospital, affiliated to Shiraz University of Medical Sciences in Southern Iran, and 34 healthy individuals. The blood samples were prepared in the morning of the day before the surgical operation. The final diagnosis of SCC was confirmed by histopathological examination of biopsied specimens. The healthy control group was enrolled the volunteer individuals without any positive history of known inflammatory or infectious diseases. Also, OSCC patients with a past history of chemotherapy, radiotherapy or surgery, as well as other malignancies or mentioned diseases above, were excluded. All the study subjects signed consent form. Stage and grade of tumors were obtained from patients' medical files. Those data were not available, were not considered in statistical analysis.

MCT analysis

The blood samples were centrifuged at 4˚c and the sera were stored at -80˚C until use. We used an ELISA Kit (Hu 8934, Biotang Company, USA) with sandwich ELISA method, according to the manufacturer's instructions.

Data were analyzed by SPSS (version 11) using T-test, Mann- Whitney and correlation coefficient tests. P- value<0.05 was considered as significant.

Results

The OSCC patients were 55 individuals (29 males, 26 females) with mean age of 63.9±15 years. 64% of patients were smokers. The available clinicopathologic data of the patients including histopathological grade and TNM stage parameters were illustrated in Table 1. The control group included 34 healthy subjects (20 males, 14 females) with mean age of 60.8±16 years.

Table 1. Data of Patients' Stage and Tumor Grade

		Number	Percent
Tumor size	T_1	7	12.7
	T_2	30	54.5
	T_3	11	20
	T_4	2	3.6
Regional Lymph Node status	N_0	19	34.5
	N_1	20	36.4
	N_2	11	20
	N_3	1	1.8
Distant metastasis	M_0	49	89.1
	M_1	2	3.6
TNM Stage	I	1	1.8
	II	11	20
	III	25	45.4
	IV	17	30.9
Histopathologic Grade	I	25	45.5
	II	11	20
	III	3	5.4

Table 2. Serum Mast Cell Tryptase (MCT) Level in the Groups

Group	Number	MCT Level	
		Range	Mean±SD
Patients	55	0-70.7	12±14
Control	34	0-33.5	8.5±9.1

The mean of serum level of MCT was 12±14 ng/ ml in the OSCC group and it was 8.5±9.1 ng/ ml in the control group. (Table 2) Mann-Whitney test did not show any significant difference between two study groups. Also, there was not a significant correlation between MCT and tumor grade, stage, location and smoking status (PV>0.05).

However, MCT level showed positive linear correlation with tumor size (P=0.04, r= 0.3). The mean of MCT was 13.3±17.5 ng/ ml in males and 10.5±8.5 in females, but T-test displayed that this difference was not significant (PV>0.05).

Discussion

For the first time, in 1891, Westple described accumulation of mast cells around the tumors (Coussens et al., 1999). Thereafter, this phenomenon has been confirmed in many studies. MCT level in biologic fluids is used as an indicator of number and activation of mast cells (Hogan and Schwartz, 1997). This level has been determined in breast and pancreatic cancer (Samoszuk and Corwin, 2003; Strouch et al., 2010). Strouch et al. have stated that mast cell accumulation in the tumoral tissue may be reflected in the serum and the serum level of tryptase may be a useful marker to distinguish malignant from benign tumors (Strouch et al., 2010).

According to the results of previous studies that have revealed increased mast cell density in OSCC tissue, we evaluated MCT in OSCC patients' sera .We found that MCT level increased in the serum, but there was no significant difference between this level in the patients

and healthy individuals. The normal tryptase level has been recorded to be below 10-15 ng/ml, depending on the referral laboratory (Valent et al., 2011). The greater levels indicated mast cell activation. We observed a mean of 12±14 ng/ml in the patients' sera which was only a little more than the normal range. Samozuk and Corwin have shown a mean of 10.3±2.4 in women with different stage of breast cancer, which was three times greater than normal women (Samoszuk and Corwin, 2003). Sperr et al. have also demonstrated elevated level of tryptase (greater than 15 ng/ml) in 40-44% of acute myeloid leukemia (AML), but not in acute lymphocytic leukemia (ALL) patients (Sperr et al., 2001). Moreover, patients with malignant pancreatic cancer have shown an elevated tryptase level compared to the benign tumors in the same location (Strouch et al., 2010).

The method of measurement was different in the above studies, while we used sandwich ELISA, Samozuk and Sperr et al. have used commercial fluoroenzyme immunoassay (FIA) in breast cancer and leukemia patients, and Strouch et al. have used chemicon quantitative spectrophotometery (Sperr et al., 2001; Samoszuk and Corwin, 2003; Strouch et al., 2010). It seems that all of these methods had sufficient sensitivity for determination of total tryptase level.

Although, the serum MCT level in our patient group was comparable with the previous studies, our healthy control group had a greater tryptase level. Normal subjects in the previous study had a mean of 3-5 in comparison to 8.5±9.1 in our samples (Sperr et al., 2001; Samoszuk and Corwin, 2003). It has been shown that serum tryptase may increase in some conditions, including allergic/atopic disorders, some types of leukemia, mastocytosis, Helminth infection, as well as end-stage of kidney diseases (Valent et al., 2011). Also, idiopathic elevation of MCT may be found in some healthy individuals (Ruëff et al., 2010). With a limited number of healthy control group in our study, it is possible that some of these healthy individuals had occult allergic or inflammatory diseases despite the complete clinical history we have taken. This probably has lead to an increase in the tryptase mean.

Our findings in patients' serum did not reflect the results of other studies on oral and lip SCC tissues that have shown significant increased mast cell count in tumoral specimens (Iamaroon et al., 2003; Jaafari-Ashkavandi et al., 2010; Tinge et al., 2010). However, some authors have demonstrated decreased mast cell density in OSCC tissue compared to normal oral mucosa (Oliveira-Neto et al., 2007; Cheema et al., 2012).

According to our results, there was no correlation between tryptase level and histopathological grade, clinical stage, patients nodal status and metastasis, these correlations have not been described in the previous reports that have studied patients' sera in other malignancies. Moreover, other studies that have been done on tumoral tissues have revealed an obvious controversy. The mast cell count has had positive correlation with tumor grade in pancreatic cancer and OSCC, while some researchers have shown that high-grade SCC were not presented with mast cell accumulation (Coussens et al., 1999; Strouch et al., 2010; Cheema et al., 2012).This controversy may be due to the difference in tumor cell to stroma ratio among various neoplasms and different methods of mast cell counting.

We observed a positive linear correlation between tryptase level and tumor size. This result was predictable, because mast cells accumulate around the tumors and larger tumors are surrounded by a larger peripheral area. Based on the patients' gender, slight- but not significant elevation of tryptase levels was found in males. Males with pancreatic cancer and BCC have shown greater mast cell count in the previous researches (Tayebi-Meybodi et al., 2007; Valent et al., 2011).This finding has been attributed to the increased sun exposure in males with BCC (Tayebi-Meybodi et al., 2007), however more investigations should be designed to determine any association between gender and mast cell quantity and activity.

In conclusion, according to the results of the present study serum level of MCT in OSCC patients, unlike breast and pancreatic cancer, is not a useful diagnostic and prognostic marker. Employing this marker as a certain paraclinical parameter may require ruling out any source of inflammation and allergy which difficulties inevitably difficult in the routine screening of the patients.

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