Evaluation of the Serum Level of High Mobility Group Box 1 Protein in Benign and Malignant Salivary Gland Tumors

Maryam Mardani[1,2], Azadeh Andisheh Tadbir[1,3], Sadaf Pourshahian[2], Bijan Khademi[4], Mahyar Malekzadeh[5]

[1] *Oral and Dental Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[2] *Department of Oral and Maxillofacial Medicine, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran*

[3] *Department of Oral and Maxillofacial Pathology, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran*

[4] *Department of Otorhinolaryngology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[5] *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

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Abstract- Despite a low prevalence, salivary gland tumors (SGTs) represent a diverse set of tumors with a broad range of biological behaviors. Implementation of early detection programs has significantly improved the outcome of treatment and patients' survival. High mobility group box one protein (HMGB1) may likely be a candidate for the detection of SGTs due to its background in other human tumors. This study, for the first time, aimed to investigate the clinical value of HMGB1 in patients with benign and malignant SGTs and analyze its correlation with clinicopathologic outcomes. Using an enzyme-linked immunosorbent assay (ELISA), the serum level of HMGB1 was measured in 85 patients with SGTs (30 benign and 55 malignant cases) and 85 age- and sex-matched healthy individuals. HMGB1 levels had a significant difference between patients with SGTs and healthy controls (2041.4±787.1 pg/ml versus 536.3±374.6 pg/ml, P<0.0001) as well as those with benign and malignant tumors (1680.1±429.7 pg/ml versus 2238.6±867.2 pg/ml, P<0.0001). The serum level of HMGB1 was associated with some clinicopathologic factors, such as the size of the main tumor, clinical stage, and the lymph node metastasis, but not with patients' gender, age as well as the site of the lesions. These results suggest that the serum level of HMGB1 has the potential to be a supportive diagnostic marker for SGTs and can provide a precise assessment of the tumor status. There is no published report regarding the serum level of HMGB1 in SGTs; therefore, further studies are warranted.

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Keywords: Salivary gland tumors; High mobility group box one protein (HMGB1); Clinicopathologic factors

Introduction

Salivary gland tumors (SGTs) are one of the relatively uncommon groups of human lesions encountered in oral pathology practice at any age and represent 3-6% of all head and neck neoplasms. There are most complex and come in a variety of benign and malignant forms (1-3). The incidence of SGTs in the Iranian population has been estimated to be 0.4-4.9% (4-6). Due to its infrequency, the prolonged risk of recurrence, complex histopathological diagnosis, the grade of malignancy, and varied clinical behavior, the management of salivary gland neoplasms is challenging for both the patients and clinicians. However, the outcome of patients with SGTs has improved in recent years, most likely due to diagnosis at an earlier stage of the disease. Early detection of tumors plays a crucial role in successful therapy and reduces the severity of impact on the patient's life (7,3).

High mobility group box one protein (HMGB1), also known as amphoterin, is a potent proinflammatory cytokine that is actively secreted by the inflammatory cells and passively released into the extracellular milieu from the necrotic cells (8-10). It is highly conserved

Corresponding Author: A. Andisheh Tadbir
Oral and Dental Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Tel: +98 7136263193, Fax: +98 7136263193, E-mail address: andisheh@sums.ac.ir

among species and identified in the nuclei and cytoplasm of almost all cell types. HMGB1 plays a key role in intracellular and extracellular functions. Under normal conditions, HMGB1 stabilizes nucleosome formation within the cell and is involved in DNA replication, recognition of DNA damage as well as transcriptional regulation (11,12). Interestingly, an extracellular HMGB1 is closely associated with each of the hallmarks of cancer, including unlimited replicative potential, evasion of apoptosis, proliferation, angiogenesis, inflammation, malignant tumorigenesis, tissue invasion, and development of metastasis. All these are related to the ability of HMGB1 to bind to its major membrane receptor, called the receptor for advanced glycation end products (RAGE) (13-15,8,16). The role of RAGE in tumor progression and metastasis was described previously (17,12).

In recent years, accumulating evidence has strongly indicated the overexpression of HMGB1 in various types of malignancies such as breast, prostate, liver, colorectal, and head and neck squamous cell carcinoma (8,18,16,19-22); however, little information is available about its serological activity. There is no published report regarding the clinical value of HMGB1 in patients with SGTs; therefore, the current study aimed to investigate the serum level of HMGB1 in Iranian patients with benign and malignant SGTs to explore the prognostic value and its relationship with the clinicopathological features of SGTs.

Materials and Methods

Study population

A total of 85 patients with SGTs who were routinely visited in the ENT Department of Khalili Hospital, affiliated to Shiraz University of Medical Sciences (SUMS), Shiraz, Iran, were enrolled in this cross-sectional study. The patients' group consisted of 30 benign and 55 malignant cases. Tumor-node-metastasis (TNM) staging was defined according to the international staging system for SGTs (www.cancer.org). The patients' inclusion criteria were being pathologically proven benign or malignant SGTs, which received no treatment before blood collection. All patients with evidence of tumors from other tissues except the salivary gland as well as those with active systemic or an inflammatory disease were excluded. Medical records were obtained from the patients' database. Besides, 85 age- and sex-matched healthy individuals without any systemic or inflammatory disease were included as a control group. The protocol of this study was reviewed and approved by the local Ethics Committee of SUMS (IR.SUMS.REC.1397.16). Written informed consent was obtained from all subjects, and the patients' privacy was ensured.

Evaluation of the HMGB1 level

Before any treatment, 5 ml of blood samples were collected in non-heparinized tubes and allowed to clot at room temperature for half an hour, then centrifuged at 3000 RPM for 5 min. Sandwich enzyme-linked immunosorbent assay (ELISA) was used for evaluation of the serum levels of HMGB1 by a commercially available kit (MyBioSource, USA) following the manufacturer's guidelines. The optical density (OD) of the wells was measured at 450 nm in a microplate reader (Biochrom Anthos 2020, Cambridge, UK).

Statistical analysis

All statistical analyses were carried out using SPSS version 15 (SPSS, Chicago, USA). Variables were tested for normal dispersion by the Kolmogorov-Smirnov test. Continuous variables were expressed as the mean ± standard deviation (SD). Comparisons between the study groups were made by one-way analysis of variance (ANOVA) with a post-hoc Tukey test. Pearson's χ^2 test was used for assessing differences between categorical variables. A P less than 0.05 was considered to be statistically significant.

Results

Eighty-five patients with pathologically proven SGTs, including 37 male (43.5%) and 48 female (56.5%), were enrolled in the current study. Their ages ranged from 12 to 83 years (mean age=49.4 years, SD=17.4). No significant differences were observed between the patients and the control groups with regards to age and sex (P>0.05). Patients were divided into two groups of benign (n=30) and malignant (n=55) subjects with lesions in the parotid, submandibular, sublingual, and minor salivary glands. The demographic and clinical characteristics of the SGT patients are summarized in Table 1.

Serum HMGB1 levels in patients with SGTs

The mean value of serum HMGB1 in patients with SGTs was significantly elevated in comparison to healthy controls (2041.4±787.1 pg/ml versus 536.3±374.6 pg/ml, P<0.0001). The levels of HMGB1 were significantly different between patients with benign (1680.1±429.7 pg/ml) and malignant

(2238.6±867.2 pg/ml) SGTs (*P*<0.0001). Moreover, there was a significant difference in the mean serum HMGB1 levels between both benign or malignant SGTs and healthy controls (*P*<0.0001 and *P*<0.0001, respectively). However, it was not associated with the patients' gender and age (*P*>0.05).

Relationship between the serum HMGB1 levels and clinicopathologic factors

A positive correlation coefficient of 0.487 was found between the serum HMGB1 levels and the size of the main tumors (*P*<0.0001). The mean serum HMGB1 level in tumors larger than 4 mm was significantly higher than the tumor of less than 4 mm (2484.3±904.7 pg/ml versus 1716.1±481.6 pg/ml, *P*<0.0001). The site of the lesions did not affect the serum HMGB1 levels; however, all the parotid, submandibular, sublingual, and minor SGTs had a higher level of HMGB1 compared with the healthy controls (*P*<0.05). There was a significant correlation between serum HMGB1 levels and the TNM stage (Figure 1).

The mean value of serum HMGB1 was 1184.6±224.1 pg/ml, 1762.9±355.9 pg/ml, 2219.3±878.2 pg/ml, and 2704.9±811.2 pg/ml in patients at TNM stage I, II, III, and IV. HMGB1 levels between healthy controls and those in stage I (*P*=0.005), as well as stage I and stage II (*P*=0.029), stage II and stage III (*P*=0.014), and stage III and stage IV (*P*=0.025), showed statistically significant differences. Furthermore, the serum level of HMGB1 was significantly different between patients with and without lymph node metastasis (4112.4±129.5 pg/ml versus 1965.7±690.8 pg/ml, *P*<0.0001).

Table 1. Demographic and clinical characteristics of the patients

Variables		Benign SGTs* n=30	Malignant SGTs n=55	Total n=85
Sex, Male (%)		14 (46.7%)	23 (41.8%)	37 (43.5%)
Age (mean±SD)		47.6 ± 18.5	50.4 ± 16.9	49.4 ± 17.4
Size of the main tumor (mean ± SD)		3.6 ± 1.1	4.4 ± 2.4	4.2 ± 2.1
Location of lesion	**Parotid glands**	22 (73.3%)	37 (67.3%)	59 (69.4%)
	Submandibular glands	8 (26.7%)	5 (9.1%)	13 (15.3%)
	Sublingual glands	N/F**	2 (3.6%)	2 (2.4%)
	Minor glands	N/F	11 (20%)	11 (12.9%)
TNM stage	**I**	2 (6.7%)	47 (12.7%)	9 (10.6%)
	II	21 (70%)	13 (23.6%)	34 (40%)
	III	7 (23.3%)	15 (27.3%)	22 (25.9%)
	IV	N/F	20 (36.4%)	20 (23.5%)

*SGTs: salivary gland tumors
**N/A: not found

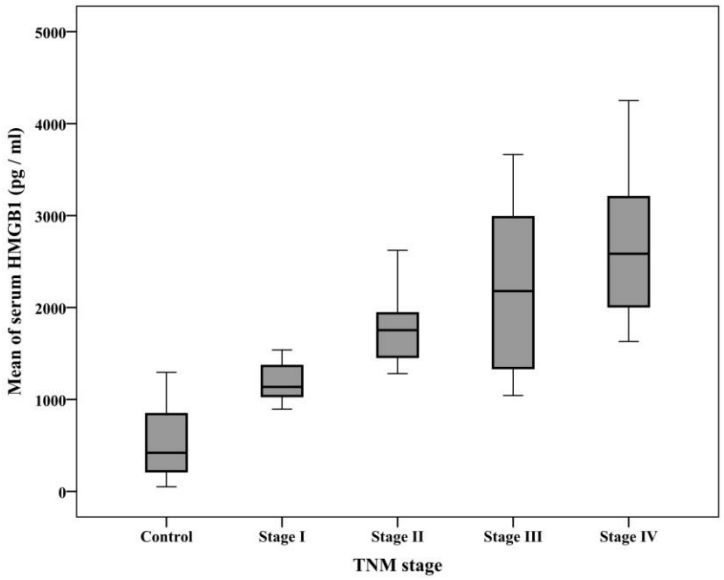

Figure 1. Comparison of the serum HMGB1 levels in different TNM stages. Values between healthy controls and those in stage I (*P*=0.005), as well as stage I and stage II (*P*=0.029), stage II and stage III (*P*=0.014), and stage III and stage IV (*P*=0.025), showed statistically significant differences

Discussion

Most of the studies conducted on HMGB1 were evaluated its levels in tissues. Although the histological examination is the gold standard for the diagnosis of tumors, there are expensive and not without risk for the patients. Therefore, research interests have focused on non-invasive methods such as blood-based testing to improve the differential diagnosis of neoplasms and management of cancer patients (23-25). HMGB1 can be passively released into the extracellular space or serum of patients during various pathogenic processes. It is overexpressed in human cancers and strongly correlated with the development of malignancies, angiogenesis, and tumor metastasis (26,27,12,11).

In the present study, the potential of HMGB1 as a biomarker for the detection of SGTs was evaluated. We demonstrated the higher levels of HMGB1 in the serum of the patients' group in comparison to healthy controls. Currently, there are no published data regarding the role of HMGB1 in salivary gland neoplasms. However, in line with our results, almost all previous studies have shown the elevation of HMGB1 in the sera of patients with various types of human tumors, such as laryngeal squamous cell carcinoma (28), cervical squamous cell carcinoma (29), head and neck squamous cell carcinoma (30), colorectal carcinoma (31), hepatocellular carcinoma (32), breast cancer (8), lung cancer (11), and gastric cancer (25). However, our data are in contrast to those who reported a similar level of HMGB1 in the serum of patients with epithelial ovarian cancer (33). The increased amount of HMGB1 observed in our series of patients suggests it as a supportive diagnostic marker for SGTs.

Despite the detection of HMGB1 in the sera of patients with different cancers, its clinical value and correlations were poorly understood. A comparison of the serological activity of HMGB1 in patients with SGTs suggests its clinical significance to differentiate between benign and malignant cases. We identified that the concentration of serum HMGB1 was higher in patients with malignant SGTs than in benign subjects. This is in agreement with those who reported a higher level of HMGB1 in patients with breast cancer in comparison to benign breast disease (8). Furthermore, the results of this study showed that HMGB1 was strongly correlated with tumor stage. Elevated levels of HMGB1 are associated with either the inflammatory process of the disease or tumor cell death; however, differentiating these conditions in clinical samples was not possible with the current tools. The marked increase in serological activity of HMGB1 at TNM stage I might be linked to immune activation from the early-stage cancers, while its elevation in stage IV might be linked to the tumor development and malignant transformation (31,32). Our results are consistent with that reported in patients with lung cancer (11), gastric cancer (25), colorectal carcinoma (31), ovarian cancer (34), and hepatocellular carcinoma (32). However, Wild *et al*., (30) could not detect any significant associations between the serum level of HMGB1 and the TNM stage or the other clinicopathologic characteristics of patients with head and neck cancer.

Besides, HMGB1 might be useful for the prediction of lymph node involvement. The serological activity of HMGB1 increased in the samples of SGT patients with lymph node metastasis, which is in agreement with those reported in patients with gastric cancer (25) and laryngeal squamous cell carcinoma (28). Overexpression of HMGB1 has been strongly correlated with tumorigenesis, invasion, and metastasis via binding to RAGE and activation of key signaling pathways. Moreover, an enhanced level of HMGB1 may cause modulation of the transcriptional expression of other genes involved in neoplasm progression and metastasis; therefore, it seems that HMGB1 can serve as a target for the prevention and treatment of various types of tumors (35,32,36,37). The suppression of tumor growth and metastasis was also observed following RAGE-HMGB1 complex blocking in a mice model (37,38). Furthermore, HMGB1 could be an oncoprotein that contributes to the formation and development of tumors; therefore, it could be highly secreted during early-stage cancers, implying that HMGB1 will be helpful for the diagnostic approaches, particularly in earlier stages (31). Finally, no significant association was found between the serum level of HMGB1 and patients' sex and age which is compatible with the previous reports (16,28).

The present study showed that HMGB1 could be considered as a valuable serological biomarker for the diagnosis of SGTs at an earlier stage of the disease. Our findings confirm that HMGB1 plays an important role in the development of SGTs, and its level was positively correlated with the tumor size and TNM stage. Therefore, targeting HMGB1 production or its release might have therapeutic potential for SGTs. Although these preliminary results support the clinical value of serum HMGB1, further studies are warranted to assess its potential roles in SGTs and other oral malignancies.

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