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ORIGINAL RESEARCH

Prognostic New Marker (Bone Morphogenetic Protein 7) in Squamous Cell Carcinoma

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ABSTRACT

Aim: Despite advances in the treatment of oral squamous cell carcinoma (OSCC), its prognosis is still poor. Therefore, it is important to identify the prognostic factors. The aim was to investigate the level of bone morphogenetic protein 7 (BMP7) in SCC of tongue and its relationship with some clinicopathologic parameters.

Materials and methods: In this cross-sectional study, 128 patients with primary SCC of tongue were evaluated. Data were extracted and paraffin blocks were retrieved from the archives of Emam Khomeini Hospital, Tehran, and immunohistochemistry staining was done for the detection of marker. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 16 and through chi-square and logistic regression tests.

Results: The highest level of BMP7 expression was 54% in men ($p = 0.044$), 70% in the group aged under 45 years ($p = 0.001$), 68.2% in patients with lymph node metastasis ($p < 0.001$), and 100% in those with poorly differentiated tumors ($p < 0.001$). In multivariate analysis, the presence of lymph node metastasis and increased histopathological grade associated with 5.7 fold and 4.3 fold increase in the odds ratio (OR) of BMP7 expression respectively.

Conclusion: Based on the results, there was a significant relationship between BMP7 expression and poor cellular differentiation and lymph node metastasis, so this marker could be a new prognostic marker in oral cancer.

Clinical significance: This new marker could help clinicians to determine the prognosis of oral cancer, so it has an effect on optimal treatment.

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INTRODUCTION

Oral squamous cell carcinoma is the most common head and neck malignant tumor and usually happens among aged people; however, in recent years, its incidence among young people has increased noticeably.[1,2] The increased incidence can be attributed to the higher level of several risk factors, such as human immunodeficiency virus, human papilloma virus infection, family history, smoking, marijuana abuse, alcohol consumption, and air pollution.[3,4]

Surgery is the best choice of treatment for this cancer; however, the rate of recurrence after treatment is about 32% and the average time of recurrence is about 14 months.[5] The 5-year survival rate in patients with this type of cancer varies from 17 to 80% based on cancer location (local or metastatic).[6] Therefore, finding a reliable marker for the recurrence, metastasis, and prognosis of disease among the affected patients can help to treat them more effectively and can improve their survival rate.[7,8]

Bone morphogenetic proteins, which is a member of the family of transforming growth factor-β, plays important roles in regulating cell proliferation, apoptosis, cell differentiation, migration, and invasion. It is also involved in a wide variety of processes.[9,10] The BMP7 is observed in a variety of tumors, such as breast, prostate, esophagus, and colon; it plays a major role in the regulation of cell differentiation in these tumors.[11-14] Two studies showed

that BMP7 had more expression in cancerous cells of esophageal SCC in which they led to more metastasis; therefore, it can be used as a factor for prognosis of the disease.[9] Moreover, one study showed that inhibition of this marker in tumor cells could decrease their invasion. As a result, it may suggest a new way of treatment[9]; however, there is still limited number of studies on the subject.

In the last decade, many markers have been investigated in relation to various cancers that identifying these markers can be used to prevent, determine prognosis, and accelerate the use of therapeutic interventions. Therefore, this study was aimed to investigate the incidence rate of BMP7 in SCC and its relationship with some clinicopathologic parameters.

MATERIALS AND METHODS

In this descriptive cross-sectional study, 140 patients with primary SCC of tongue who were referred to the Cancer Institute of Imam Khomeini hospital, Tehran, for the pathological examination were studied. However, 12 patients were excluded from the study due to incomplete information records and inappropriate paraffin blocks. After obtaining informed consent from the patients and explaining the objectives of the study, we used the data registered in the medical and pathological records. Inclusion criteria were: (1) confirmation of diagnosis "SCC of the tongue," (2) primary tumor, (3) not having a history of chemotherapy or radiology before the surgery, and (4) previous history of undergoing neck dissection for the study of lymph nodes, and (5) the presence of other concomitant lesions. Exclusion criteria were: small and insufficient samples for microscopic slide preparation.

Demographic data including age and sex of the patient and pathological data of the tumor, such as histopathologic differentiation and lymph node metastases status were extracted from patients' records. The paraffin blocks of the desired samples were retrieved and in case of insufficiency of the amount of the remaining tumor tissue, a 4-µm cut section was taken from the blocks to study the incidence of marker (BMP7) (human monoclonal antibody, R&D system), using immunohistochemistry stain. Immunohistochemistry was performed according to the manufacturer's instructions, antibodies with EnVision method on paraffin blocks. Hematoxylin and eosin-stained microscopic slides were separately collected and by using an optical microscope (OLYMPUS, CX31, Japan), the diagnosis of SCC and its histopathological grades (well differentiated, moderately differentiated, and poorly differentiated) was determined. With regard to lymph node involvement, the samples were classified into two groups of lymph node metastasis (N positive) and without lymph node metastasis (N negative).

To assess the incidence rate of BMP7, 10 fields were selected in the invasive front of the tumor, and then in each field, 100 cells were counted with a magnification of 400×. In microscopic evaluation, BMP7 immunoexpression in tumor cells was assessed semi-quantitatively in terms of percentage and intensity and final immunoreactivity score. The percentage of stained cells to the total cells was calculated and the incidence was defined as 0 to 29% = 1, 30 to 59% = 2, and >60% = 3. The incidence intensity was defined in comparison with the adjacent normal tissue as negative = 0, weak = 1, moderate = 2, strong = 3. The two values obtained for the intensity and the percentage of incidence in each slide were multiplied by each other. In the final evaluation, we used the numbers greater than or equal to four as high expression and numbers less than four as low expression.[12] For positive control, prostate adenocarcinoma was used. For negative control, the monoclonal primary antibody was substituted for phosphate-buffered saline.

The SPSS version 16 software was used for data analysis. Chi-square test and Fisher's exact test were used to marginally assess the correlation between the independent clinicopathological variables in the form of categorical variables with each of the dependent variables. Logistic regression was used to eliminate the influence of confounding variables. The significance level was determined as less than 0.05.

RESULTS

The mean and standard deviation of participants' age was 56.08 ± 16.04 (age = 26–93 years). Of all participants, 78 patients (60.9%) were male (Table 1). Considering the percentage of stained cells, in 32 patients (25%), 60% or more were stained, in 60 patients (46.9%), 30 to 59% were stained, and in 36 patients (28.1%), 0 to 29% were stained. Considering the intensity of staining, 14 cases (10.9%) had strong staining, 61 patients (47.7%) had weak staining, 45 patients (35.2%) had moderate staining, and 8 people (6.3%) had negative staining. Of all, 66 patients (51.6%) had lymph node metastasis and 66 patients (51.6%) had poorly differentiated tumor. Overall, 73 patients (57%) had BMP7 expression.

The incidence of BMP7 was significantly associated with sex (p = 0.044) and the percentage of the incidence of marker was higher in samples taken from women than men (54 *vs*. 36%). Age in this study was classified into three categories: ≤44 years, 45 to 64 years, and ≥65 years. Nearly 70% of the samples were positive in terms of high incidence in the age group ≤44 years. Also, there was a significant relationship between age and BMP7 expression (p = 0.001). In 68.2% of those with lymph node metastasis

Table 1: Comparison of BMP7 expression based on different variables

Variable	*Group*	*BMP7 expression*		*Sum*	*Significant level*
		Low	*High*		
Sex	Male	50 (64.1%)	28 (35.9%)	78 (100%)	0.044
	Female	23 (46%)	27 (54%)	50 (100%)	
Age group	≤44 years	10 (30.3%)	23 (69.7%)	33 (100%)	0.001
	45 to 64 years	37 (65.8%)	17 (31.5%)	54 (100%)	
	≥65 years	26 (63.4%)	15 (36.6%)	41 (100%)	
Lymph node metastasis	No	52 (83.9%)	10 (16.1%)	62 (100%)	<0.001
	Yes	21 (31.8%)	45 (68.2%)	66 (100%)	
Histopathologic differentiation grade	Well differentiated	40 (83.3%)	8 (16.7%)	48 (100%)	<0.001
	Moderately differentiated	33 (50%)	33 (50%)	66 (100%)	
	Poorly differentiated	0 (0%)	14 (100%)	14 (100%)	

Table 2: The relationship between BMP7 expression with tumor differentiation grade and lymph node metastasis by controlling for age and sex

Variable	*Beta test statistic*	*Standard error*	*Wald test*	*Significance level*	*OR*	*95% CI for the OR*	
						Lower limit	*Upper limit*
Sex	0.204	0.480	0.180	0.671	1.226	0.479	3.140
Lymph node metastasis	1.756	0.473	13.764	<0.001	5.788	2.289	14.632
Histopathologic differentiation grade	1.468	0.434	11.466	0.001	4.341	1.856	10.154
Age	−0.013	0.015	0.772	0.379	0.987	0.959	1.016

and in 16.1% of those without metastasis, overexpression of BMP7 was observed ($p < 0.001$); 100% of people with poorly differentiated tumor had BMP7 overexpression, but the percentage of patients with well differentiation grade was 16.7% ($p < 0.001$) (Table 1).

In the multivariate analysis, there was no significant relationship between marker expression and gender ($p = 0.671$) and age ($p = 0.379$); however, grade and lymph node metastasis retained their significant association. By matching the effect of age, gender and grade, having a lymph metastasis with a 5.7-fold increase is associated with the incidence of BMP7 index [OR = 5.78; 95% confidence interval (CI): 2.28–14.63; $p < 0.001$]. Also, by matching the effect of age and sex and lymph metastasis, each degree of increase in grade with an increase of 4.3 is associated with the incidence of BMP7 index (OR = 4.34; 95% CI: 1.85–10.15; $p = 0.001$) (Table 2).

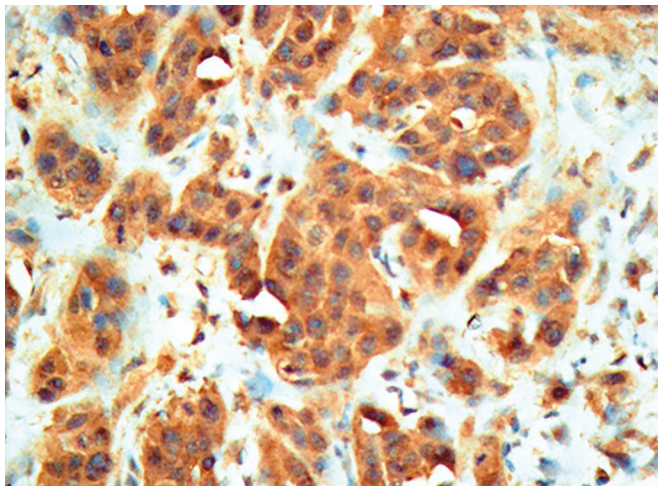

Fig. 1: The BMP7 overexpression in the cytoplasm of tumor cells (400×)

DISCUSSION

Based on the results of this study, in univariate analysis, overexpression of BMP7 was observed (Fig. 1) in lower ages, among females, in patients with metastasis, and in those with poor tumor differentiation. However, after adjusting the effects of age and sex, in multivariate analysis, the OR of lymph node metastases had an increase of 5.7-fold and with every grade of increase, BMP7 increased by 4.3-fold.

The BMP plays various roles in regulating cell proliferation, apoptosis, cell differentiation, and human evolution and they are involved in a wide variety of processes.[9,10]

No study has been conducted on the incidence of BMP7 in OSCC, and it seems that the present study is the first study on this subject. Therefore, the results cannot be compared with the results of other similar studies. However, few studies have been published on the relationship between these markers with other types of cancer. In a study on the second stage of esophageal SCC, patients with BMP7 expression had more tendencies to metastasize to the lymph nodes. In that study, BMP7 expression was associated with patients' survival and lifetime.[9] In another study, overexpression of BMP7 was associated with decreased time to recurrence of the disease and the marker was introduced as a prognostic marker for patients with cutaneous melanoma.[15]

A large variation in the level of BMP expression in breast cancer has been observed. The BMP signals can affect the cell behavior and BMP7 has a wide and powerful expression in breast cancer.[16] In Chen et al study,[17] a significant change in cell stimulation and agglutination was observed which was dependent on BMP7 concentration. In their study, low levels of BMP7 were associated with longer survival. They concluded that BMP7 has an important role in controlling the stimulation of lung cancer cells and regulation of motility and agglutination of cells; moreover, due to the inverse relationship between BMP7 expressions with lymph nodes metastasis, the impact of the marker on the gene expression of the tumor can play a therapeutic role in lung cancer.

Another study showed that messenger ribonucleic acid (mRNA) BMP7 expression in colorectal tumor tissue was significantly greater than that in nontumor tissues; in addition, mRNA BMP7 expression was associated with the depth of tumor invasion, liver and lymph node metastasis, liver recurrence, and cancer-related mortality. This study demonstrated that mRNA BMP7 alone and independent of other factors can be a predictor of the poor prognosis[18] which is consistent with the results of our study. Another study also showed that tumors with higher expression of BMP7 have a higher potential of malignancy and this marker can be used as an effective prognostic marker for gastrointestinal cancer.[12] In Aoki et al[19] study, BMP7 expression at the cell membrane in 55% of cases of gastric cancer was positive and it was significantly associated with the diameter of the tumor, node involvement, lymph node invasion, age, gender, and tumor depth. The survival rate was significantly lower in BMP7 overexpression. Masuda et al[20] studied prostate cancer samples, and prostate cancer metastasis was diagnosed in 71% of lesions and BMP7 expression was significantly high; it showed that overexpression of BMP7 was associated with cancer metastasis.

The BMP7 can alter the metastasis status through its impact on the expression of some genes associated with metastasis. Its impact might be due to changes of matrix metalloproteinase-9 (MMP-9) and E-cadherin in invasive tumors.[9] The BMP binding to receptor types I and II results in SMAD phosphorylation and leads to the activation of genes which can control proliferation, differentiation, metastasis, and apoptosis.[21] It is also mentioned in other studies that the likely mechanism of metastasis is so that BMP7 leads to increased expression of E-cadherin and MMP-9, and accordingly, it leads to increased cell migration and metastasis from esophageal SCC.[9] However, the role of BMP in the escalation of tumor genesis and metastasis in cancers is not clear yet[22] and every day, more evidences in favor of their involvement are discovered.

One of the strengths of our study was the control of confounding factors. In the multivariate analysis, after adjusting the effects of age, sex, and grade, lymph node metastasis was associated with a 5.7-fold increase in the OR of BMP7 marker (OR = 5.78; 95% CI: 2.28–14.63; $p < 0.001$). Moreover, after matching the effects of age and sex and lymph node metastases, with every unit of increase in the grade, the OR of BMP7 marker had a 4.3 fold increase (OR = 4.34; 95% CI: 1.85–10.15; $p = 0.001$). In fact, a comparison of the simple marginal analysis with adjusted analysis in multivariate models showed that the relationship between age and sex with BMP7 was altered and confounded, but the relationship between the incidence of marker with the grade and metastasis was probably real. Hence, it can be concluded that both a higher grade (poor cellular differentiation) and lymph node metastases are associated with higher ORs for incidence; moreover, the association is independent of sex and age and also independent of each other.

Overall, the results of this study and other studies may suggest a new treatment for OSCC. Inhibition of this marker in tumor cells can lead to decreased invasion and therefore can likely put forward a new treatment method.[9] Nevertheless, further investigations on human cases are required.

CONCLUSION

Based on the results, BMP7 was associated with a poor differentiation of tumor cells in lingual SCC; poor differentiation indicates BMP7 overexpression. In addition, in tumors that had lymph nodes metastasis, BMP7 was also overexpressed. The relationships between BMP7 and tumor differentiation and lymph node metastasis were independent of age and gender. It means that the overexpression of BMP7 can be a prognostic factor for the disease.

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