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Original Research

Analysis of influencing factors and prognosis of early postoperative recurrence, secondary tumor and metastasis of oral squamous cell carcinoma

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Abstract: Oral squamous cell carcinoma (OSCC) is one of the most common cancers in the world and it accounts for more than 90% of oral cancers. In this study, we tried to estimate the risk of early postoperative recurrence, secondary tumor and metastasis of OSCC to predict the patient's prognosis according to its clinical condition to help increase their survival by screening high-risk patients. 153 patients with OSCC who were over 40 years of age were studied during 1985-2020. The influencing factors included gender, race, stage of tumor progression, treatment method, histological grade and tumor location, date of diagnosis and death, which were analyzed by the Markov multi-state model. Also, their saliva was sampled to determine the amount of Matrix Metalloproteinase13 (MMP13). Following-up of patients for 60 months showed that one year after the end of treatment, the probability of death was almost the same for patients with early postoperative recurrence or secondary tumor, but after 5 years, patients with early postoperative recurrence are at higher risk of death. Also, the MMP13 amount in the saliva of patients showed that high levels of MMP13 belonged to metastasis of OSCC than early postoperative recurrence and secondary tumor. Therefore, patients with more amount of MMP13 are more involved in metastasis than early postoperative recurrence and secondary tumor. Approximate knowledge of OSCC patients' next state and time according to their clinical condition can be one of the ways of timely diagnosis and treatment and thus reduce their mortality rate.

Key words: Matrix Metalloproteinase13; Metastasis; Oral squamous cell carcinoma; Prognosis; Recurrence; Secondary tumor.

Introduction

Cancer is one of the leading causes of human mortality (1-5). The world's population is getting older, and the incidence of cancer is directly related to aging, therefore, the number of people with cancer is increasing. Five percent of all tumors occur in the head and the neck, which half of them occur in the oral cavity (6). Cancers in this area are one of the leading causes of death and ugliness worldwide and they are considered as one of the 10 most common cancers among humans (7). Squamous cell carcinoma with more than 90% incidence is one of the most common malignancies among head and neck cancers (8). One of the important causes of mortality in these patients is early postoperative and cervical recurrence. The recurrence risk of tumors in these areas is relatively high compared to cancers in other parts of the body (9). According to various studies, the rate of local and cervical recurrences has been 19-34%, and patients with cervical recurrence will have a poorer prognosis than other patients (10). There are different results regarding the local recurrence and prognosis of these patients, including Schwartz *et al.* (11) reported that the tumor recurrence period was a reasonable factor for the survival of these patients, while in another study, the effect of the tumor recurrence period was no statistically significant difference in patient survival (12). But with these interpretations, despite recent advances in treatment methods, the mortality rate of a significant proportion of these patients with early postoperative recurrence, development of systemic metastasis, and secondary tumors are still high (13).

Early detection using screening programs is one of the important goals of health officials to control and reduce the risk of serious consequences such as death in some chronic progressive diseases (14). Oral cancers are also among these diseases, which early detection and timely action play an important role in reducing mortality in this group of patients (15). In patients with squamous cell carcinoma, screening and diagnosis of high-risk patients for recurrence, secondary tumor or metastasis and the development of appropriate follow-up courses can also be effective (16). Because these patients may experience recurrence of the tumor in different ways after treatment, the Markov multi-state model can be a useful way of describing the process by which a person moves between different postures over time. Because when the event is more than one desired outcome, separate analysis of events, in addition to having low power, but also the correlation between events is not considered, which in itself will cause skew in the results. Multi-state models analyze data by considering death as the result of the presence of one or more intermediate events; In addition, by using it, the effect of predictor variables on the patient's transition can be obtained from one situation to another (17-19).

Recently, tumor markers have been increasingly used, helping to detect Oral Squamous Cell Carcino-

ma (OSCC) in the very early stages, even before the slightest clinical symptoms appear in the patient. These markers are specific proteins and mRNAs in saliva and serum that changing in their amount can indicate OSCC in a person (20, 21). Matrix Metalloproteinase (MMPs) are a group of enzymes that are responsible for breaking down the extracellular matrix and play a key role in phenomena such as proliferation, differentiation, apoptosis, angiogenesis, morphogenesis, and tissue repair. The basement membrane, which separates the epithelium from the mesenchymal tissue, is the first barrier against tumor spread. Decomposition of basement membrane and extracellular matrix requires the activity of MMP(22). MMP gene expression and activity in normal body tissues are usually low, but in cases such as pathological changes that lead to tissue destruction, inflammatory diseases, tumor growth, and metastasis, MMP activity is very high (23) (23). So far, 25 members of the MMP family have been identified, which MMP13 being identified as an important marker for OSCC determination (22, 24).

In this study, an attempt has been made to analyze influencing factors and to estimate the risk of early postoperative recurrence, secondary tumor and metastasis using multi-state model analysis, as well as to predict the patient's prognosis according to its clinical condition, so that it can be screened by high-risk patients and planning appropriate follow-up courses to help in timely diagnosis and selection of appropriate treatment strategies to increase the survival of OSCC patients.

Materials and Methods

In this study, 153 oral squamous cell carcinoma (OSCC) patients were studied who were older than 40 years old and were admitted during 1985-2020. The median follow-up was 60 months. Information about these patients includes gender, race, date of birth, stage of tumor progression, histological grade and tumor location, type of treatment plan, dates of diagnosis and death. Patients with metastatic cancer at the first diagnosis and patients without early postoperative recurrence, secondary tumor or metastasis were excluded from the study. The collected data were analyzed by R software version 3.1.2., and a significance level of 0.05 was considered.

Markov multi-state model

Figure 1 identifies five possible states 1. Alive with local recurrence, 2. Early postoperative recurrence, 3. Secondary tumor, 4. Metastasis, and 5. The death that each patient at time *t* may be in *S(t)* state. The direction of the arrows in Figure 1 indicates the path and the direction of transition from one state to another. State (5) will be the state of absorption; So that when the patient is in this state, it will not be possible to move or transfer to other states. The patient's next state and transfer time are controlled by a set of transfer intensities for each pair of status *r* and *s*, which generally depend on the individual transfer set or time-dependent variable *z*(*t*). The intensity of transitions indicates the immediate risk of transition from r to s and is defined as follows:

Δt is a small-time interval that leads to zero. Then all the values of the transfer intensity will be in the form of a *Q* matrix in which the sum of rows is zero. Therefore, the diagonal values of this matrix $\{q_{rr} = -\sum_{s \neq r} q_{rs}\}$ are based on the fit of the multi-state model to the data (25).

Saliva sample

The "Spitting" method was used to prepare a non-stimulating saliva sample. For this purpose, patients were asked to abstain from eating, drinking, and brushing for 90 minutes before sampling. Samples were collected between 9 am and 11 am. The patient emptied the saliva into the sterile test tube every 10 minutes for 2 times while sitting and relaxing, slightly forward. After coding and sealing the tube with parafilm, it was centrifuged for 10 minutes at 2000 rpm.

ELISA Test

To evaluate salivary cytokines, the linked enzyme kit (immune sorbent assay) ELISA Eastbiopharm sandwich antibody double Biotin technique was used. In this method, MMP13 was added to wells previously coated with "monoclonal MMP13 antibody". It was then incubated, and in order to bind to "HRP-Streptaridine," MMP13-Antiantibody (labeled as biotin) was added to the wells. Wells B&A Chromogen and "Stop" solution were added.

The color change created in the wells due to the empty well was read as zero control and reference. The color change showed the amount of MMP13 in each sample. Reagents, samples and standard solution were prepared first. Then the second antibody (labeled as biotin), and the ELISA solution were prepared to the sample and the standard solution was added, at 37 °C, for 60 minutes. The plates were washed 5 times (wash step), B&A Chromogen was added, incubated for 10 minutes at 37 °C to obtain a color change. Then the stop solution was added and the amount of "OD" was read

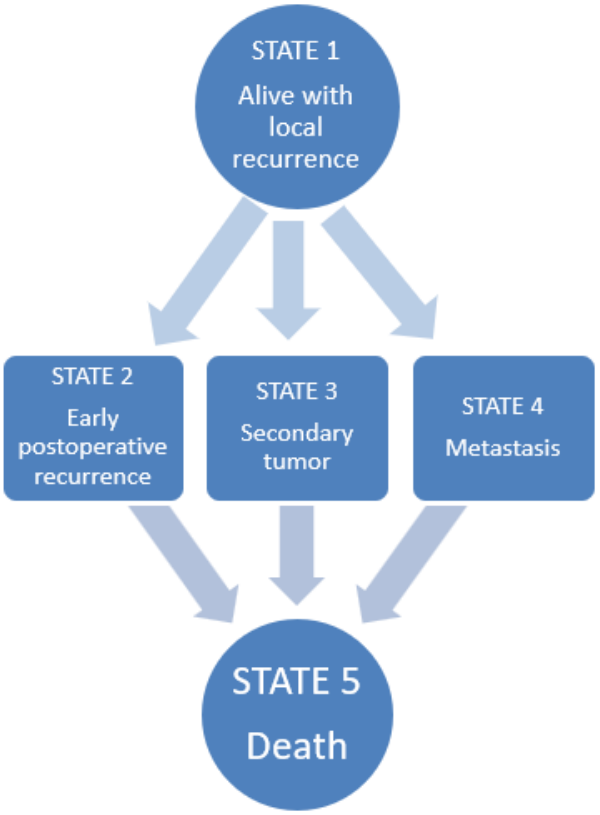

Figure 1. Diagram of cancer progression by Markov multi-state model.

10 minutes later. Finally, the obtained data were entered into SPSS software and analyzed by Square-Chi and T-tests and Pearson correlation coefficient at a significant level of $p<0.05$.

Results

From the 153 patients studied in this study, 92 (60.13%) were men and the rest were women. Among these patients, 54 cases (35.29%) early postoperative recurrence, 127 cases (84.31%) secondary tumor, 25 cases (16.99%) metastasis and 63 cases (41.17%) death occurred (Table 1). The treatment programs applied to patients are divided into two categories: surgery and postoperative radiotherapy.

In the process of OSCC, patients are faced with 5 possible states defined in Figure 1. In this study, the set of patient transfers between these 5 states was analyzed by Markov multi-state model.

Table 2 shows the estimation of transfer probability for each of the variables by patient transfer status. As shown in this table, the probability of transmission to the secondary tumor in patients whose cancerous tumor is associated with lymphatic invasion is 1.37 (95% confidence interval: 1.05-2.05) times more than those patients whose tumor was diagnosed at an early stage, and the probability of death is 2.33 (95% confidence interval: 1.29-4.18) times more among them. Squamous cell tumors with a histological grade of one have 2.66 times (95% confidence interval: 1.14-5.30) higher risk of recurrence than a histological grade of two; While its secondary tumor is up to about one-third less likely to die. Background variables and treatment methods did not make a significant difference in patient status.

Table 3 considers the likelihood of the patient changing status after two specified periods of 5 and 1 years. The most likely condition after one year for a patient in state 1 is to remain in the same condition, but after 5 years the probability of the patient's death increases significantly. Within a year, the risk of death is almost the same for a patient with an early postoperative recurrence or secondary tumor, but after 5 years, a patient with an early postoperative recurrence is at greater risk of death.

Table 4 calculates the probability of the patient's next status for each state relative to the average time interval between transfers. As can be seen, the patient who is in state 1 is transferred to state 3 with a probability of 0.65 to the other two states and the patient who is in state 3, the possibility that the next condition he/she experiences is state 5 more likely than other states.

The results of the ELISA test from patients' saliva showed that the average amount of MMP13 in OSCC patients with metastasis was the highest (8.42± 2.34ng/ml). Then patients with secondary tumors with a rate of 7.89±2.11ng/ml were ranked second. The lowest rate belonged to early postoperative recurrence (7.21±1.90 ng/ml) (Table 5).

Discussion

Early postoperative recurrence, secondary tumor and metastasis are very important cases that play a major role in the evaluation and prognosis of oral cancer and even other cancers, so that they are the most important causes of death among these patients (15). Therefore, it is very important to study and determine the factors and patients who are more prone to these risks, but due to the problems related to the evaluation of various aspects of the factors affecting this risk and the complexity of statistical analysis methods, less attention is paid to these aspects. Searches among the articles showed that the use of the Markov multi-state model has been very rare in cancer studies, especially oral cancers. Although accurate information is required for multi-state models in large volumes of samples with long follow-ups, instead, high statistical power will be available with accurate and reliable results (26). As the results of this study confirmed, although gender factor is effective in head and neck cancers, it does not make a statistically significant difference in death due to early postoperative recurrence or metastasis (27). Race is another factor that has been linked to oral cancer in various studies. According to the latest SEER database, white men and women

Table 1. Frequency distribution of patients for each of the factors by gender.

		Gender	
Factors		**Men**	**Women**
Tumor location	The floor of the mouth	12	9
	Gums and other parts of the oral cavity	28	19
	Throat	25	7
	Lips	3	3
	Salivary glands	2	0
	Tongue	22	23
Race	Black	82	49
	White	7	8
	Others	3	4
Tumor histologic grade	1	13	18
	2	57	32
	3	22	11
Tumor progression stage	1 and 2	49	37
	3	43	24

Table 2. Estimation of transfer probability using Markov multi-state model.

Factors		State Changing 1→2 Probability of transfer	1→2 95% confidence interval	1→3 Probability of transfer	1→3 95% confidence interval	1→4 Probability of transfer	1→4 95% confidence interval	2→5 Probability of transfer	2→5 95% confidence interval	3→5 Probability of transfer	3→5 95% confidence interval	4→5 Probability of transfer	4→5 95% confidence interval
Gender (women)	Men	0.77	(0.41-1.49)	0.78	(0.32-1.19)	1.86	(0.51-6.83)	0.92	(0.25-3.33)	1.27	(0.67-2.39)	2.37	(0.61-9.17)
Race (Black)	White	2.86	(0.39-20.98)	0.73	(0.37-1.41)	0.90	(0.11-7.05)	0.53	(0.04-6.04)	1.45	(0.33-3.26)	- ¥	-
Tumor progression stage (1,2)	3	0.61	(0.30-1.23)	1.37	(1.05-2.05)*	1.72	(0.57-5.13)	2.15	(0.54-8.46)	2.33	(1.29-4.18)*	1.63	(0.37-7.13)
Tumor histologic grade (2)	1	2.66	(1.14-5.30)*	0.79	(0.44-1.43)	0.91	(0.19-4.27)	0.28	(0.04-1.85)	0.37	(0.12-0.98)*	-	-
	3	1.44	(0.63-3.27)	0.91	(0.35-1.49)	0.58	(0.12-2.67)	2.76	(0.70-10.85)	1.47	(0.72-2.98)	-	-
Treatment (surgery + radiotherapy)	Surgery	1.38	(0.71-2.67)	1.03	(0.68-1.54)	1.10	(0.37-3.27)	0.72	(0.24-2.15)	0.56	(0.30-1.00)	1.92	(0.49-9.09)

Groups in parentheses are references. ¥ Transmission probability could not be calculated (data shortage). * Significance less than 0.05.

Table 3. Matrices for estimating the probability of patient transfer between different states for a certain period.

The current state of the patient	Changing of status after 1 year 1	2	3	4	5	Changing of status after 5 years 1	2	3	4	5
1	0.17	0.06	0.16	0.02	0.03	0.19	0.08	0.32	0.03	0.37
2	0.00	0.63	0.11	0.04	0.21	0.00	0.12	0.16	0.00	0.71
3	0.00	0.24	0.74	0.03	0.20	0.00	0.04	0.30	0.01	0.67
4	0.00	0.21	0.21	0.66	0.05	0.00	0.02	0.40	0.19	0.39
5	0.00	0.00	0.00	0.00	1	0.00	0.00	0.00	0.00	1

Table 4. Matrix for estimating the probability of the patient's next state.

The patient's primary state	The patient's next state 1	2	3	4	5
1	0.00	0.26	0.65	0.08	0.00
2	0.00	0.00	0.00	0.00	0.52
3	0.00	0.00	0.00	0.00	0.74
4	0.00	0.00	0.00	0.00	0.06

Table 5. Comparison of saliva MMP13 levels between the groups of OSCC patients with early postoperative recurrence, Secondary tumor, Metastasis (ng/ml).

	Early postoperative recurrence Mean±SD	Secondary tumor Mean±SD	Metastasis Mean±SD	P-value
MMP13 amount	7.21±1.90	7.89±2.11	8.42± 2.34	0.002

have a higher incidence of cancer than blacks, but these rates vary with mortality; So that for white and black women this rate is almost the same; but, on the other hand, Black men have higher mortality rates than whites (28). Kademani *et al.* (29) reported that the patient's race was ineffective in their survival, which confirms some of the findings of this study. No study was found on early postoperative recurrence, metastasis or secondary tumor and its relationship with patients' race. One of the other important factors is the effect of the disease progression stage (30). As a result, patients who are in advanced stages are significantly more at risk for secondary tumors and death than patients with early-stage tumors, but this effect was not observed in relation to early postoperative recurrence. A similar study by Sklenicka *et al.* (31) did not report the effect of tumor progression stage on early postoperative recurrence. In contrast, another study reported this effect as significant (31). In confirming the results of this study, the study of Sun *et al.* (32), which was performed on a secondary tumor of the tongue, reports the effect of the tumor progression stage is significant on the survival of patients. The histological grade of the tumor is another factor that can be effective in the prognosis of the disease. Although most researchers believe that the information provided by this triple classification is insufficient and weak, some studies report the effect of histological grade on early postoperative lymphatic recurrence (33-35). One of the significant findings in this study was grade 1 squamous cell tumors are riskier for early postoperative recurrence than grade 2. But grade 2 Secondary tumors are more likely to die than grade 1, but as mentioned, different results have been reported in different studies, which can be one of the reasons why most researchers believe in the poor prognosis for the histological grade of the tumor. In comparing the risk of early postoperative recurrence, secondary tumor and metastasis in the presence of different factors, no significant difference was found between treatment methods (36). For example, in the study by Liu *et al*.(10), there was no difference between surgical and non-surgical methods, or in the study by Brandwein *et al*.(37), it was reported the effect of postoperative radiotherapy on recurrence and long-term survival only for high-risk patients. From these findings, it can be inferred that radiotherapy should be limited to patients as much as possible because it causes patients to face significant technical limitations such as dosage and toxicology in case of early postoperative recurrence (38). As mentioned, the high incidence of secondary tumors has the greatest effect on the survival rate of these patients. Identifying patients and high-risk intervals will be one of the methods for timely diagnosis and action for patients. In a 5-year follow-up study for secondary tumors in patients with squamous cell carcinoma of the lower lip, Van Der Tol *et al.* (39) reported that follow-up of high-risk patients with secondary tumors is best done for 5 years at approximately 2.7 times a year.

In relation to MMP13, Vincent-Chong *et al.* (40) showed that 95.5% of tissue samples of OSCC patients had an increase in the expression of the MMP13 gene. Also, the difference between MMP13 protein in normal epithelium and OSCC was statistically significant. In a study by Agha-Hosseini *et al*. (41), the mean increase of MMP13 in the saliva is higher than the mean increase in serum. This suggests that saliva could be more reliable for measuring MMP13 in patients with OSCC, as we did in the current study. The study by Marcos *et al.* (42) also showed that the presence of high levels of MMP13 in the salvia and serum of squamous cell carcinoma patients is associated with the possibility of lymph node metastasis. They determined the sensitivity of MMP13 as a tumor marker to be 76% and its specificity to be 100%. Also, they stated that MMP13 has a "good" diagnostic value for determining OSCC disease, while other MMPs, including MMP9 and MMP2, do not have this characteristic. In the present study, results showed that patients with more amount of MMP13 are more involved in metastasis than early postoperative recurrence and secondary tumor. Therefore, according to the significant difference in MMP13 levels among OSCC patients with metastasis compared to early postoperative recurrence and secondary tumor, it seems that this enzyme can be a helpful diagnostic criterion for determining the presence of this disease.

A comprehensive study of the genome, as well as gene expression, is important in the study of diseases and disorders (43, 44). There is a need for further studies on the analysis of effective factors and prognosis of postoperative recurrence, secondary tumor and metastasis for various cancers (45-47).

In the current study, using the features of the Markov multi-state model and the output of Tables 3, 4 and 5 useful information was provided for the prognosis of different patients at different stages of OSCC progression.

Various factors are involved in treatment failure and the occurrence of early postoperative recurrence, secondary tumor and metastasis in patients with oral cancer. In the current study, in addition to examining patients, according to various influencing factors, it was tried to find a model for predicting the next states of the Oral Squamous Cell Carcinoma (OSCC) patient so that timely diagnosis and treatment can be done.

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