Evaluation of Clinical, Demographic, Pathological, and Molecular Factors with Survival Rate of Patients with Oral Squamous cell Carcinoma in Yazd city During 1998–2008

Original Article

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Abstract

Introdouction:

Squamous cell carcinoma is the most common oral cancer and the prognosis because of a late diagnosis remains poor despite numerous treatments. Therefore, we conducted a cross-sectional study to investigate the relationships between survival rate(SR) of oral squamous cell carcinoma (OSCC) and clinical,demographic, pathological, and molecular factors in Yazd city during 1998–2008.

Materials and methods:

Data related to 30 Yazdian patients with OSCC who were referred to Shahid Sadoughi Dental School and Hospital during 1998–2008 were evaluated according to census data. Clinical and histopathological data were gathered via phone calls, and archived specimens were immunohistochemically stained to examine the cell proliferation index (Ki-67), the anti-apoptotic index (bcl2), and a tumor suppressor indicator(p53). The data were analyzed using SPSS statistical software (V.17) via a Kaplan–Meier analysis, and p <0.05 was considered significant.

Results:

Eighteen cases (60%) were females and 12(40%) were males. The mean 5-year SR was lower in men than women and in patients >50 years of age than <50 years, the mean SR from lowest to highest were record to labial, lingual and intraoral involvement respectively and for smokers and patients with a moderate disease grade(II) and intense p53 staining tended to be lower than other categories; however, the differences were not significant. The overall 5-year SR of patients was 55% in this study, and the mean survival was 6.6 years.

Conclusion:

The SR was lower in older men and smokers Therefore, a more radical treatment and longer follow-upafter treatment for older male smokers are recommended.

Key words:

•Carcinoma •Squamous Cell •Mouth Mucosa •Tumor Suppressor Protein p53 •Survival Rate

Introduction

Cancer is a genetic disease affected by many environmental factors.[1] Oral cancer accounts for 2%–4% of all malignancies, although the prevalence in some countries is higher, such as 10% in Pakistan and 45% in India[2] Squamous cell carcinoma (SCC) is the most prevalent cancer occurring in the oral cavity and constitutes >90% of oral malignancies. The incidence of this cancer increases with age, particularly in males >40 years of age.[3] Alcohol and tobacco are the most common etiological factors for oral squamous cell carcinoma (OSCC).[4] Approximately 275,000 new cases of OSCC are reported worldwide each year.[5] Despite the advances in therapeutics and diagnosis, the overall prognosis of OSCC remains unfavorable, with 30% recurrence rates from local or regional disease, 25% for distal metastases, and a 40% 5-year survival rate.[6]

The association between the OSCC prognosis and age and sex is controversial, as some studies have not shown such a relationship,[7, 8] whereas others have revealed that the prognosis of OSCC is worse in older aged men.[9, 10] No relationship between prognosis and smoking and alcohol use was reported in one study,[7] whereas another reported higher mortality rates in cigarette smokers and alcohol drinkers.[11] A study indicated that there was no relationship between prognosis and disease grade,[12] whereas another study reported higher mortality in patients with higher disease grades.[13]

Various studies have found no correlations between high Ki-67, bcl-2, and p53 expression and prognosis,[14-16]) whereas other studies have reported that high Ki-67, bcl-2, and p53 expression is related to a poor prognosis.[17-19]

Considering these controversies, we conducted this study to investigate the associations between survival rate and clinical, pathological, and molecular factors in patients with OSCC in Yazd, Iran.

Materials and Methods

This cross-sectional study was designed to investigate some survival rate indices of patients with OSCC. The patients were referred to the Dental School of Shahid Sadoughi University of Medical Sciences during 1998–2008. At least 5 years had passed since their cancer diagnoses, and all patients had undergone similar therapeutic procedures including radical surgery and radiotherapy.

Inclusion and Exclusion Criteria: Patients with incomplete records, insufficient paraffin block sample volume, those who had died due to anything except OSCC, and those treated with a different mode of therapy were excluded from the study.

The patient's clinical information was obtained through telephone calls and included age, sex, smoking history, and site of mouth involvement (involvement of the tongue or other intraoral area and labial sites). Pathological data, such as disease grade, were gathered from the patient's medical records and microscopic slides available in the pathology archive were reviewed.

Of the 30 OSCC samples investigated, 12 were grade I, 14 were grade II, and four were grade III (Table 1). Immunohistochemical staining for the Ki-67, bcl-2, and p53 markers was performed on the paraffin blocks of the available biopsy specimens as a molecular examination.

Immunohistochemical Investigation: The paraffin blocks were sectioned at 2 μm thickness, deparaffinized in an oven, and dehydrated in an alcohol series. The sections were immersed in Tris-buffered saline (TBS) (Sigma-Aldrich, St. Louis, MO, USA) at pH 7.4 for 10 min. Hydrogen peroxide (1: 10 in methanol; DakoCytomation, Glostrup, Denmark) was used to block non-specific staining for 20 min in a dark moist environment. The sections were rinsed with water, and the antigens were retrieved in an EDTA citrate buffer (D-6100; Merck, Stockholm, Germany). The tissue sections were placed in a microwave oven at maximum power(100 °C - 20 min)and then at one-third power for 20 min. The proteins were blocked for 20 min after producing a hydrophobic barrier around the tissue.

Antibodies to Ki-67 (clone MIB1l; DakoCytomation), bcl-2 (clone 124; Dako North America, Carpentaria, CA, USA), and p53 (clone Do-7; DakoCytomation) were added to the sections. After 40 min, the sections were rinsed with water and immersed in TBS (pH 7.4). Then, the Envision horseradish-peroxidase substrate (Ready-to-use, DakoCytomation) was added to the sections for 30 min. The sections were rinsed

twice with TBS (pH 7.4), and 1 ml chromogen substrate was applied. The sections were rinsed again with TBS solution and then with water. The specimens were stained with hematoxylin, dehydrated with xylol and alcohol, mounted, and labeled. The prepared slides were observed under a light microscope by two pathologists. The number of nuclei that stained brown was counted, and the labeling index was determined .

Based on the percentage of stained nuclei, the samples were classified as follows: <5%, negative; 5–25%, weak; 25–50%, medium; and >50%, intense [20] (Figures 1–4).

Data were analyzed with the Kaplan–Meier analysis using SPSS 17 software (SPSS Inc.

Chicago, IL, USA). $P < 0.05$ was considered significant.

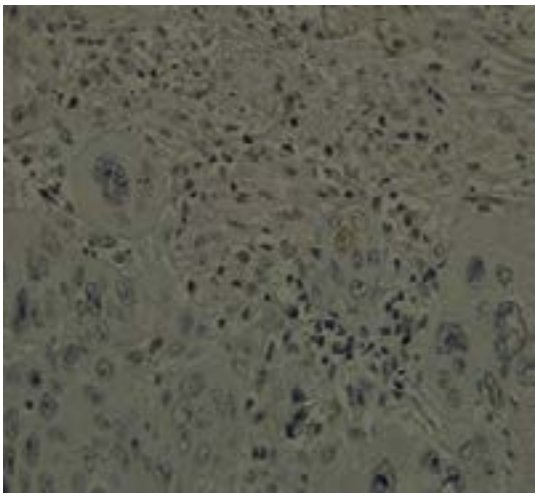

Figure 1: Negative staining for the p53 marker (magnification, ×40).

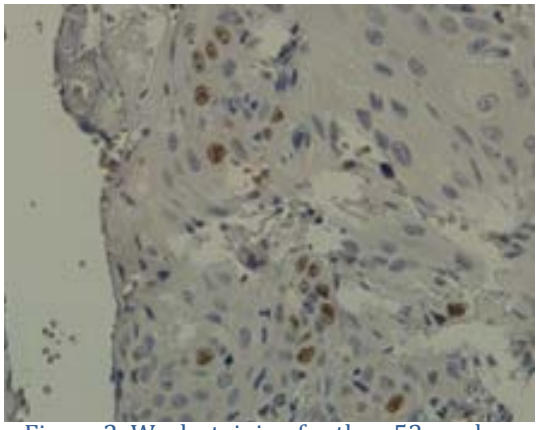

Figure 2: Weak staining for the p53 marker (magnification, ×40).

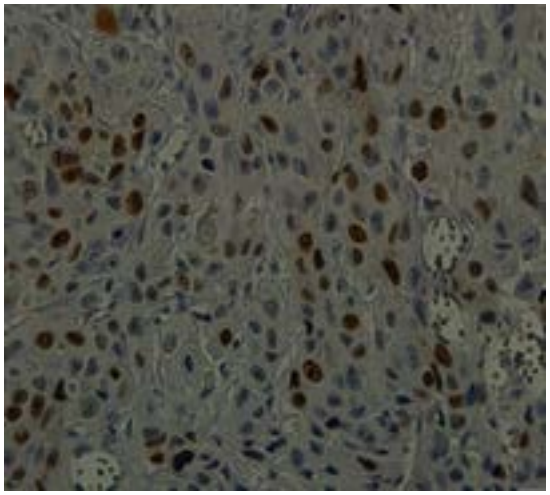

Figure 3: Medium staining for the p53 marker (magnification, ×40).

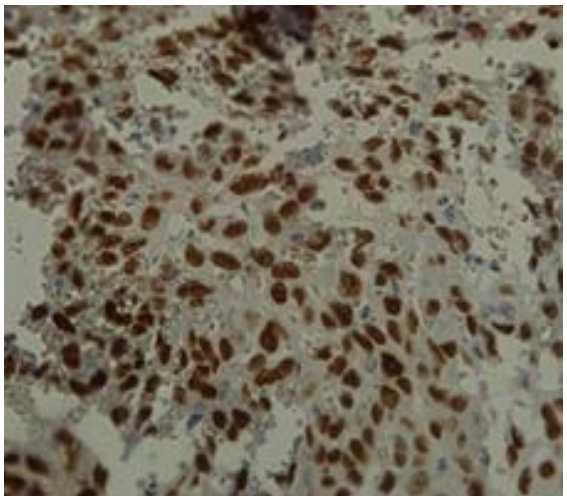

Figure 4. Intense staining for the p53 marker (magnification, ×40).

Results

The subjects included 18 women (60%) and 12 men (40%) with a mean age of 66.33 ± 13.44 years (Table 1).The results of this study indicate that women were more affected by the disease than men, and the mean overall survival rate was lower in men than that in women. However, no statistically significant difference was found ($P > 0.05$) (Table 1).

The overall 5-year survival rate was 55% with a mean survival rate of 6.6 years. The 5-year survival rates were 56% in women and 47% in men. Patients more than 50 years of age had a higher prevalence and shorter mean survival time than those less than 50 years of age, which was not statistically significant ($P > 0.05$) (Table 1).

The highest prevalence of lesions by anatomic

site occurred on the tongue, followed by the mouth and lips. Mean survival times from lowest to highest were related to labial, lingual, and intraoral involvement, respectively, which was not statistically meaningful (P > 0.05) (Table 1).
Non-smokers tended to have a higher prevalence of OSCC than that in smokers; however, the mean survival time for smokers was lower than that for non-smokers.
This difference was not statistically significant (P > 0.05) (Table 1).
Most patients had grade II disease, followed by grades I and III. Mean survival time for patients with a moderate disease grade (II) was lower than that of patients with other grades; however, no statistically significant difference was observed (P > 0.05) (Table 1).
The histological samples of most patients stained negative for p53 (40%), followed by medium (23%), intense (20%), and weak staining (16.7%). The mean survival rate of patients with intense p53 staining tended to be lower than that of patients with the other categories of p53 staining (Table 1).
Approximately 94% of the samples negatively stained for bcl-2, and 6.7% expressed weak bcl-2 staining. Therefore, no survival rate analysis could be conducted (Table 1).
Most patients (60%) showed weak Ki-67 staining intensity, followed by medium (20%), negative (16.7%), and intense (3.3%). No survival rate analysis was performed, as no death was reported in any case of negative Ki-67 staining (Table 1).
The subjects included 18 women (60%) and 12 men (40%) with a mean age of 66.33 ± 13.44 years (Table 1).The results of this study indicate that women were more affected by the disease than men, and the mean overall survival rate was lower in men than that in women. However, no statistically significant difference was found (P > 0.05) (Table 1).
The overall 5-year survival rate was 55% with a mean survival rate of 6.6 years. The 5-year survival rates were 56% in women and 47% in men. Patients > 50 years of age had a higher prevalence and shorter mean survival time than those< 50 years of age, which was not statistically significant (P > 0.05) (Table 1).
The highest prevalence of lesions by anatomic site occurred on the tongue, followed by the mouth and lips. Mean survival times from lowest to highest were related to labial, lingual, and intraoral involvement, respectively, which was not statistically meaningful (P > 0.05) (Table 1).
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Most patients (60%) showed weak Ki-67 staining intensity, followed by medium (20%), negative (16.7%), and intense (3.3%). No survival rate analysis was performed, as no death was reported in any case of negative Ki-67 staining (Table 1).

Discussion

More women (60%) than men (40%) with OSCC were included in this study; therefore, the female: male involvement ratio was 1.5: 1. Similar to our result, Delavarian et al.[21] reported a 1: 0.7 ratio of women with OSCC to men. In contrast, Eshghyar et al.[22] found more males than females with OSCC and reported a ratio of 5: 4. The ratio in the present study does not represent the incidence ratio of the two sexes in Yazd city but rather suggests the population ratio of patients referred for treatment.
In the present study, the 5-year overall survival rate was 55%, with a mean length of life of 6.6 years and the 5-year survival rate was 56% in women and 47% in men. Lo et al.[7] reported no difference in survival rate for men and women

Table 1: Comparison of survival rates according to individual patient characteristics

variants		Frequency (%)	Mean and Standard Deviation	P-value
sex	female	18(60)	7.18 ±0.75	0.099
	male	12(40)	5.87 ±0.75	
age	50<	8(26.7)	6.66 ±1.2	0.766
	50≤	22(73.3)	6.56 ±0.66	
Involvement site	tongue	15(50)	6.72 ±0.76	0.586
	Other sites	12(40)	6.70 ±1.03	
	lips	3(10)	4 ±0	
smoking	Yes	5(16.7)	4 ±0	0.120
	No	25(83.3)	6.86 ±0.60	
Disease grading	I	12(40)	6.33 ±0.71	0.221
	II	14(46.7)	5.33 ±.60	
	III	4(13.3)	9 ±0	
P53 protein	Negative (less than 5%)	12(40)	7.57 ±1.04	0.365
	weak (5%–25%)	5(16.7)	8 ±1.15	
	medium (25%–50%)	7(23.3)	6 ±0.91	
	intense (more than 50%)	6(20)	5 ±0	
Bcl2 protein	Negative (less than 5%)	28	0	0
	weak (5%–25%)	2	0	
	medium (25%–50%)	0	0	
	intense (more than 50%)	0	0	
Ki-67 protein	Negative (less than 5%)	5	0	0
	weak (5%–25%)	18	0	
	medium (25%–50%)	6	0	
	intense (more than 50%)	1	0	
5-years survival	live	11(36.7)	0	0
	death	19(63.3)	0	

Kaplan–Meier analysis

patients with OSCC. However, Sapp et al.[10] indicated that women have a better prognosis than More women (60%) than men (40%) with OSCC were included in this study; therefore, the female: male involvement ratio was 1.5: 1. Similar to our result, Delavarian et al.[21] reported a 1: 0.7 ratio of women with OSCC to men. In contrast, Eshghyar et al.[22] found more males than females with OSCC and reported a ratio of 5: 4. The ratio in the present study does not represent the incidence ratio of the two sexes in Yazd city but rather suggests the population ratio of patients referred for treatment.

In the present study, the 5-year overall survival rate was 55%, with a mean length of life of 6.6 years and the 5-year survival rate was 56% in women and 47% in men. Lo et al.[7] reported no difference in survival rate for men and women patients with OSCC. However, Sapp et al.[10] indicated that women have a better prognosis than that of men. In contrast, Leite et al.[11] found that survival rate was lower in women than that in men.

In our study, 73.3% of patients were more than 50 years and 26.7% were less than 50 years, Our patients more than 50 years of age tended to have a shorter survival than those less than 50 years, suggesting that the older patients died due to age, for a reason other than OSCC, or due to treatment-related complications. However, age may not be an appropriate factor for assessing survival rates in patients with OSCC.

The association between age and prognosis is controversial. Some studies have shown no relationship between these factors, including Rikardsen.[8] In contrast, Ribeiro[9] claimed that older patients had a worse prognosis for OSCC and Dohlstom[23] who found that younger patients with more invasive OSCC had a worse prognosis.

In the present study, the tongue was the most common site of involvement in 50% of all cases. The lower lip was the least involved site (3.3%). Fan[24] reported that 63% of patients had OSCC of the tongue, followed by the gums. Chang[25] Taiwan, and Krishna[26] in India reported buccal mucosa as the most frequent site of OSCC involvement.

In this study, the lowest survival rate occurred in patients with OSCC in the lips, followed by the tongue, and other sites in the mouth. This may have been due to the larger size and deeper spread of lesions in these areas.

Ashu[27] reported a 15% mortality rate in patients with lip OSCC, and survival rate was 50% in cases of lymph node lesions. However, the prognosis differed in other areas of the mouth. Chen[28] reported that patients with OSCC in the floor of the mouth, gums, and maxilla had a 5-year survival rate of 15%. The 5-year survival rates were 47% in the tongue and 53% in the buccal mucosa. Unlike the present study, Araujo et al.[29] suggested that the tongue and the floor of the mouth had the worse prognosis in patients with OSCC and also reported that lower lip lesions were better differentiated compared to those of the tongue and floor of the mouth.

About 83% of the patients enrolled in the present study had no smoking history, whereas 16.7% were current or past smokers. This might be explained by the higher frequency of women with OSCC in this study.Lo et al.[7] found no associations between survival rate and smoking and alcohol drinking, which agreed with our results. Conversely, Ribeiro [9] reported higher mortality rates in smokers and alcoholics.

Most patients in the present study had a moderate-grade histopathological severity, whereas the fewest had severe grade disease. Patients with a more severe grade lived longer than those with lower grades, which may have been because of the small sample size in each category. We found no association between prognosis and histopathological grade, suggesting that disease grade is not an appropriate prognostic factor for patients with OSCC.

This finding is consistent with Charoenrat[12] who did not find a relationship between differentiation in tumor histopathology and prognosis. However, Kosunen[30] found no association between low tumor histopathological differentiation and a poor prognosis, which was confirmed by another study.[7] In addition, tumors with a higher malignancy grade had a worse prognosis than others.

p53 protein expression was positive in 60% of our samples, suggesting that an increased incidence of this marker could reduce mean length of life, as patients with more severe grades tended to have a lower survival rate. These conflicting results were attributed to different sample sizes, methodologies, staining methods, and calculations in another study.[31] Studies by Nylander [32] on SCC in the head and neck found no associations between p53 protein expression and apoptosis, whereas Oliviera[18] linked high p53 expression with a poor prognosis.

In the present study, 83.3% of our cases expressed the Ki-67 marker weakly and only 16.7% were negative for Ki-67. As no deaths were reported in our study, this marker does not have a critical role determining the prognosis of these patients. Bettendorf et al.[14] concluded that Ki-67 staining alone cannot be used to predict prognosis in patients with oral cancer. Kim et al.[17] reported that presence of the Ki-67 bio-marker determines the worst prognosis for patients with OSCC.

About 93% of our samples did not stain for the bcl-2 marker and only 6.7% expressed bcl-2 weakly. Therefore, a survival rate analysis was not possible. Wilson[33] investigated bcl-2 expression in patients with head and neck carcinoma but found no correlation between bcl-2 expression and prognosis. However, Kato[34] reported a correlation between increased levels of bcl-2 and poor survival in patients with OSCC. Confusing results about tumor protein expression in other parts of the body have also been reported, which may be due to differences in staining technique or marker evaluation.[16] Thus, it seemed that if different grading criteria are selected, the associations with other parameters would change as well.[31]

According to the results of our study, it seems that staging based on a combination of tumor size, lymph node involvement, and distant metastasis[4] is still the main factor predicting the prognosis in patients with OSCC.

A limitation of this study was that the sample characteristics and inappropriate tissue block conditions in the hospital environment affected antigen retrieval. We also had a problem with in-

complete entry of patient clinical data into their medical records, which restricted analysis of the cases. Therefore, a more comprehensive study with a larger sample size is needed to further investigate the relationship among these factors in patients with OSCC.

Conclusion

None of the variables we investigated, including demographic factors, site of involvement, microscopic disease grade, or expression of the p53 apoptosis marker played an important role predicting survival rate in patients with OSCC. However, among these factors, males were more likely to be related to shorter length of life in the patients, therefore periodic check-ups and a longer follow-up after treatment are recommended for patients with OSCC to try to increase the survival rate.

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