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Original Article

A comparative study of histological grade and expression of Ki67 protein in oral squamous cell carcinoma in young and old patients

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ABSTRACT

Background: Oral squamous cell carcinoma (SCC) is the most common cancer of oral region. The characteristic feature of SCC is invasion of dysplastic epithelium into the connective tissue. Oral SCC is more common in old patients. It is believed that etiology and pathogenesis of SCC in young patients differ from old patients and it is related to different molecular mechanism. In this study, histopathologic differentiation and proliferation activity (by Ki67) in oral SCC lesions of young patient (<40 years) and old patients (>50 years) have been compared.

Materials and Methods: In a cohort study, Formalin Fixed and paraffin-embedded tissue sections of 20 oral SCC of young patients and 20 oral SCC of old patients were stained by H and E and immunohistochemically by biotin-streptavidin method. They were observed by two pathologists. Histological grade and Ki67 labeling index (LI) were determined. Data were analyzed by *t*-test and Mann-Whitney.

Results: In cases of oral SCC in young patients, 80% were grade I and 20% were grade II and Ki67 LI was 21/5% in this age group. In cases of oral SCC in old patients, 75% were grade I and 25% were grade II and Ki67 LI was 21/6% in this age group.

Conclusion: Histological and immunohistochemical evidence of this study show that oral SCC of young patients and oral SCC lesions of old patients didn't show any differences in histopathological differention and proliferative activity.

Key Words: Histopathological grade, immunohistochemistry, Ki67, oral squamous cell carcinoma

INTRODUCTION

Squamous cell carcinoma (SCC) is one of the most common malignancies of oral region. SCC is originated from dysplastic surface of epithelium and invades to underlying connective tissue in forms of islands and cords of tumoral cells.[1] According to cellular resemblance of their parent tissue and production of their product (keratin), SCC is classified into 3 grade (well, moderate, poorly differentiation).[2] There are different grading systems like the one introduced by Bryne [Table 1].[3] For many years grading of SCC was a prognostic criterion to predict the biologic behavior of tumor. However, clinical staging of SCC is more important than histological grading.[1,2] Oral SCC typically occurs in elderly men in 5th-8th decades of life. The incidence of oral SCC in younger patients is approximately 6% in UK.[4] However, recently, incidence of oral SCC in young patient is increasing.[4] There are some reasons to believe that etiology and pathogenesis of oral SCC in young and old patients may be different.[4-6] Alternation of cell cycle proteins contribute to the biologic behavior of cancers.[4] Ki67 is one of the mitotic indicators in proliferative activity of tumors.[7,8] Studies regarding cell cycle regulator and various histological grading, that compare the young and old patient with oral SCC are sparsely available in literature. Well and moderately differentiated were the most common grade of

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Table 1: Squamous cell carcinoma granding system (Brayane classification)

Morphologic feature	Score			
	1	2	3	4
Degree of keratinization	Highly keratinized (>50% of the cells keratinized)	Moderately keratinized (20-50% of the cells keratinized)	Minimally keratinized (5-20% of the cells keratinized)	No keratinization (0-5% of the cells keratinized)
Nuclear polymorphism	Little (>75% mature cells)	Moderately (75-50% mature cells)	Abundant (50-25%) Mature cell)	Extreme (0-25% mature cells)
Pattern of invasion	Pushing well dehneated infiltrating borders	Infiltrating solid cords bands and or strands	Small groups or cords of infiltrating cells (*n*>15)	Marked and widespread cellular dissociation in small groups and or in single cells (*N*<15)
Inflammatory response	Moderate	Moderate	Sigh	None

histological of oral SCC in young patient reported by Iype *et al*. (2000) and Iamaroon *et al*. (2004) and Prado (2007).[9-11] On the other hand, in studies carried out by Effiom (2008), poorly differentiated SCC was the most common grade of oral SCC in Nigerian young patient.[12] Siriwardena (2007) showed no differences in histological grading and proliferative activity of oral SCC in old and young patients in Srilanka.[4] Furthermore, in their study, expression of PCNA indicator (as a proliferative marker) in old patient was more than young patient.[4] Therefore, according to different results in histological grading and proliferative activity of oral SCC in young and old patients in the literature, this histological and immunohistological study was designed to evaluate the histological grade and Ki67 expression (as a proliferative marker) in patient younger than 40 years old and patient older than 50 years old with oral SCC.

MATERIALS AND METHODS

In a cohort study, paraffin embedded tissues of 20 cases of young patient (<40 years) and 20 cases of old patient (>50 years) with oral SCC were obtained from the archives in Department of Oral Pathology, Dental School, Isfahan university of medical science.

Histological analysis

All the specimens were sectioned in full-thickness of tumor and all cases were stained with H and E. The sections including, invasive front were observed by two pathologists and Bryne *et al*. classification system were used to assess the histological parameter [Table 1]. The data were analyzed statistically by Mann-Whitney *U* test.

Immunoistological analysis

In order to detect the specific antigens of Ki67 the tissues were immunohistochemically stained by Biotin-Stereptavidin method. Briefly, the main procedure was:

1. Serial sectioning (in 3-4 μm sections)
2. Deparafinization
3. Rehydration
4. Antigen retrieval.

All specimens were then placed in phosphate buffered saline and were treated 5 min in protein block solution to prevent any false staining and the specimens were then incubated for 30 min with primary antibody of Ki67 (KI67 MMI1:50) (Novocastra, Dusseldorf/ Germany).

Furthermore, the sections were exposed to Novolink polymer (RE7112) or secondary antibody for 30 min and were washed by phosphate buffer salin (PBS). Then they were incubated with diamino-banzidin for 5 min for visualization. After washing the slides, they were counter stained with H. Finally, after drying the slides were mounted.

To quantify the percentage of positive cells within the lesions for Ki67 markers and obtaining the labeling index (LI), sections were observed separately by two pathologists microscopically with × 400 magnification of Olympus light microscope. Ten non-overlapping fields (containing invasive front) were selected. A minimum of 1,000 stained cells in each field were measured and the mean of all 10 fields was calculated. Data were analyzed by *t*-test. The study protocol has been approved by the Ethical Committee of Isfahan University of Medical Sciences.

RESULTS

In the present study, we found that in young individuals (<40 years), 80% of lesions were well differentiated (grade I). Furthermore, we found that in old patients (>50 years), 75% of specimens were well differentiated (grade I) [Figure 1]. The

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immunohistochemical results of this study show that Ki67 LI was 21.5% in young patients and 21.6% in old age group [Figures 2 and 3]. According to our results, there were no significant differences in histological grading and Ki67 LI in young and old patients.

DISCUSSION

SCC is one of the most common malignancies of oral region. SCC is originated from a dysplastic surface of epithelium and invades to underlying connective tissue in forms of islands and cords of tumoral cells.[1] SCC is more common in old patient than young people. However, recently, incidence of oral SCC in young patient is increasing.[4] There is some reasons to believe

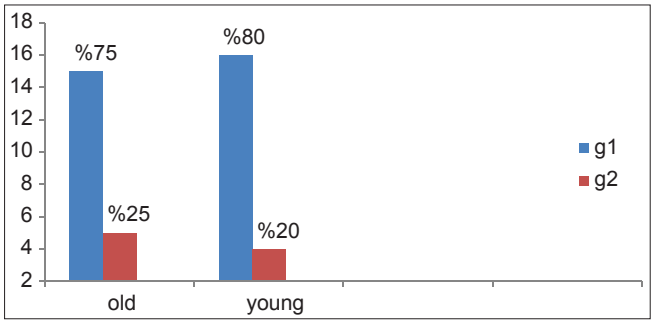

Figure 1: Histological grade of two age groups. There are no statistically differences in two groups

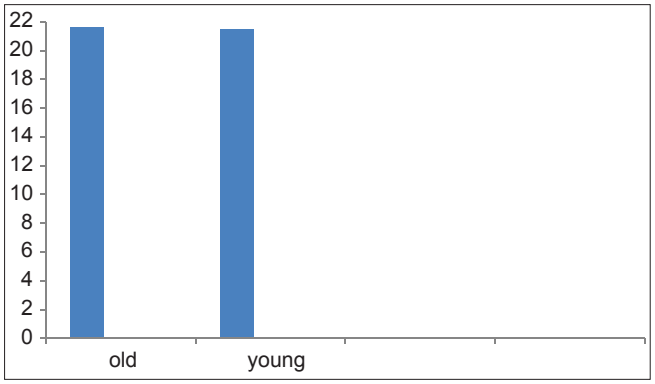

Figure 2: Ki67 labeling index of two age groups. There are no statistically differences in two groups

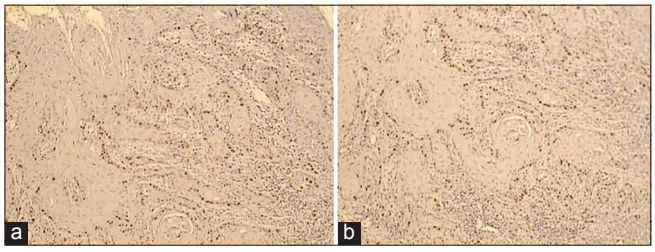

Figure 3: Ki67 expression in younger and older groups (×100). (a) young, (b) old

that etiology and pathogenesis of oral SCC may be are different between young and old patients.[13] A shorter duration of exposure to environmental carcinogens and lack of pre-existing lesion in young patients suggest the possibility of different molecular mechanisms in two groups.[4] It is long decade that histological grading and tumor differentiation use to predict the biological activity of SCC.[4] However, histological findings of oral SCC are different in various areas of tumor. It is believed that cells present at the invasive tumor front of carcinomas have different molecular characteristics when compared with superficial areas of the tumor so invasive front is most important area of the tumor for determination of prognosis.[7] Defective apoptosis and angiogenesis is important for cancer development.[14,15] Proliferative activity of tumoral cells is one of the indicators for tumoral invasion potential and invasive activity of cancers related to degree of malignant neoplastic cells.[3] PCNA and Ki67 are the most important immunohistochemical markers for proliferative activity of tumors.[7] In general, there is a controversy on clinical finding, prognosis, and degree of differentiation and proliferative activity of oral SCC in different age groups. Some investigators don't report any relation between age and those mentioned factors. Some studies suggest the worse prognosis for oral SCC in old patients, however, others believe that prognosis and clinical outcome in young patients is worse than old patients.[16] According to the importance of oral SCC in young patient, in this study, we used Bryne grading system for classifying and histological grading of oral SCC in young (<40 years) and old (>50 years) patients. We also applied Ki67 marker to determine proliferative activity of lesions in these two age groups. Results of this study shows, that there are no significant differences in histological differentiation of oral SCC in old and young patients. In this both age groups, well differentiated (grade I) was the most histopathological grade of SCC. This finding is similar to those reported by Iype *et al.* (2001) in India, Yamaroon (2001) in Thailand, and Pradoo (2009) in Brazil.[9-11] In contrast, Effiom (2009) reported the poorly differentiated carcinoma as the most common grade of oral SCC in Nigeria but in their study, Broder classification was used and they did not focus on invasive front area.

Immunohistochemical evidence of this study shows no significant differences of Ki67 LI in young and old patients. However, few studies have described correlation between Ki67 expression and age of patients with SCC. Fratazic (2008) reported no

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relation between Ki67 LI and clinicopathologic factors of oral SCC.[15] Regezi (1998) found a high level of Ki67 expression in higher grade of oral SCC[17] Radrigus (2008) reported no relation between Ki67 expression and age of patient with SCC of larynx.[18] Indeed, the results of our study confirm those reported in similar studies in other countries. The results indicate that oral SCC in young (<40 years) and old (>50 years) individuals has no differences in cell differentiation and proliferative activity. So although young patients have a shorter duration of exposure to environmental risk factors and a pre-existing lesion is rarely seen, however, histological parameters and mitotic index of oral SCC are the same as old individuals. According to less effect of extrinsic carcinogenic factors in young patients, intrinsic factors such as genetic and immunological risk-factors may have an important role. It is supposed that extrinsic and intrinsic factors act oppositely in young and old individuals. Because of this, proliferative activity of oral SCC is the same in two age groups.

CONCLUSION

Histological and immunohistochemical evidence of this study show that oral SCC of young patients and oral SCC lesions of old patients did not show any differences in histopathological differention and proliferative activity.

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