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ORIGINAL ARTICLE

Differences in oral habit and lymphocyte subpopulation affect malignant transformation of patients with oral precancer

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Background/purpose: In Taiwan, the combination of betel quid chewing, alcohol consumption, and smoking habits increases oral cancer risk by 123-fold compared to persons without these habits. Lymphocyte populations in patients may potentially affect the malignant transformation of oral precancer.
Methods: A total of 28 patients with oral precancer from our previous cohort were enrolled in this study, and their personal information and oral habits were documented. Their lymphocyte populations (CD4+, CD8+, CD19+, and CD56+) and activation markers (CD25 and CD69) were determined by flow cytometry from 1999 to 2004. After follow up till December 2014, data of

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patients with/without malignant transformation were recorded, and the relation between oral habits and percentage of initial lymphocyte markers was evaluated using the Student *t* test and Fisher's exact test.

Results: Ten precancer patients developed oral squamous cell carcinoma with a mean period of malignant transformation of 6.8 ± 2.1 years. Patients with malignant transformation had a mean age of 48.4 ± 5.0 years ($n = 10$), relatively more than that of patients without malignant transformation (41.6 ± 6.3 years, $n = 18$) ($p < 0.05$). An increase was noted in the population of peripheral blood mononuclear cells expressing CD4+CD69+, CD19+CD69+, and CD56+CD69+ ($p < 0.05$) in precancer patients with malignant transformation. Alcohol consumption showed an association with the malignant transformation of patients with precancer ($p = 0.030$), whereas betel quid and smoking showed little effect.

Conclusion: These results suggest that age, alcohol consumption, and early activation of T cells, B cells, and natural killer cells are crucial in the malignant transformation of oral precancer. Analysis of patient's lymphocyte populations may help predict the malignant transformation of oral precancer.

Introduction

In recent decades, oral cancer has become an important health problem, and it is one of the top four causes of cancer death of male in Taiwan.[1] Many studies have revealed a strong correlation between oral cancer incidence and some oral habits. In Taiwan, people with alcohol drinking, betel quid (BQ) chewing, and smoking habits have a higher risk of oral precancers, such as oral leukoplakia, oral erythroplakia, oral submucous fibrosis (OSF), and even oral squamous cell carcinoma (OSCC).[2,3] Various factors such as ethnic groups, stages of diagnosis, gender, diagnostic age, anatomic site, morphologic type, BQ chewing, and treatment methods have been shown to affect the prognosis of OSCC.[4,5] Consumption of alcohol, BQ chewing, and cigarette smoking are highly associated with the initiation, promotion, and progression of oral cancer.[6–8] However, whether these oral habits may affect the malignant transformation of oral precancer is an intriguing issue awaiting further investigation. Clinically, various therapeutic modalities such as radical surgical excision, chemotherapy, and radiotherapy, separately or in combination, have been used for the treatment of OSCC. However, the overall survival rate for oral cancer patients was 61%,[9] and the survival rates were 75%, 65.6%, 49%, and 30%, respectively, for patients with stage I, II, III, and IV oral cancer.[10] Understanding the factors responsible for the malignant transformation of oral precancer is, therefore, important for early diagnosis and treatment of OSCC, to improve the prognosis.

Chemical carcinogens in alcohol, BQ, and cigarette have been shown to affect the normal structures of protein, lipid, and DNA of oral mucosal cells, leading to gene mutation, chromosomal aberrations, and even clinical cancer.[6,7] Most of the damaged DNA can be repaired, and the transformed cells can be destroyed by the immune system. However, some transformed cells may escape the host immune surveillance, resulting in cancer development. Accordingly, recent advances in tumor immunology reveal that an imbalance between effector cells and regulatory cells in different kinds of tumor microenvironments may affect oral cancer initiation, promotion, progression, and treatment outcomes.[11,12] Immunological changes occur in different stages of oral carcinogenesis. Presence of cytogenetic damage in lymphocytes isolated from precancerous patients and impairment of cellular immune responses in cancer patients have been reported.[12,13] Alterations of lymphocyte population and functional defects in lymphocyte were noted in patients with different stages of oral cancer.[14–16] Analysis of lymphocyte phenotypes in peripheral blood mononuclear cells (PBMCs) of patients with oral precancer and cancer, therefore, can be used potentially to predict disease progression and treatment outcomes. However, limited information is known about the changes in phenotypes of PBMCs in oral precancer patients and their relation to malignant transformation.

The purposes of this study were to investigate whether the populations of CD4+ T-helper cells, CD8+ T cells, CD19+ B cells, and CD56+ natural killer (NK) cells, and their activation markers (CD25+ and CD69+) in the initial diagnostic stage of oral precancer may have an effect on future malignant transformation. Furthermore, we clarified the relationship between age or oral habits and the malignant transformation of oral precancers.

Materials and methods

By the approval of Ethics Committee, National Taiwan University Hospital, 28 consecutive male patients with oral precancerous lesion were recruited from the Department of Oral and Maxillofacial Surgery, National Taiwan University Hospital, from 1999 to 2004.[17] Patients with autoimmune diseases, those who were taking immunoactive drugs, and those with evident viral/bacterial infection within 1 month were excluded from this study. The analytic cohort consisted of 28 male patients with precancerous lesions, with a mean age of 44.0 ± 6.2 years. Basic information, and clinical and histopathological data of these patients were recorded during that period (Table 1). Expression of