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Characterization of epithelial oral dysplasia in non-smokers: First steps towards precision medicine

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ABSTRACT

Objectives: Tobacco usage is the strongest risk factor in the development of oral squamous cell carcinoma (OSCC), which mandates careful screening for oral cancers in smokers. However, there are indications that oral potentially malignant lesions, such as oral epithelial dysplasia (OED), in non-smokers (NS) have a higher cancer risk than those in smokers. Without tobacco as an etiology, the development of these lesions in NS may suggest genetic susceptibility. The increasing incidence of OSCC in NS calls for a better understanding of the natural history of OED in NS as compared to that of smokers.

Materials and methods: Patients from a population-based longitudinal study with more than 10 years of follow up were analyzed. Of the 455 patients with primary OED (233 mild and 212 moderate dysplasia), 139 were NS and 306 were smokers. Demographic and habit information, clinical information (lesion site, size and appearance; toluidine blue and fluorescent visualization), microsatellite analysis for loss of heterozygosity (LOH) and outcome (progression) were compared between the two groups.

Results and conclusions: The majority of patients with OED were smokers. Of these, more were males, non-Caucasians and heavy drinkers. A significantly higher number of OED in NS were in the tongue, whereas a significantly higher number of OED in smokers were in the floor of mouth (FOM). OED in NS showed a greater than 2-fold increase in cancer progression. Strikingly, OED located in the FOM in NS showed a 38-fold increase in cancer progression as compared to those in smokers.

Introduction

Tobacco usage is the strongest risk factor for the development of oral squamous cell carcinoma (OSCC) [1–4], which mandates careful screening for oral cancers in smokers. However, OSCC does develop in non-smokers (NS), and there are indications that oral potentially malignant lesions (OPML) in NS possess a higher cancer risk than those in smokers [5–8]. Without tobacco as an etiology, the development of these lesions in NS may suggest genetic susceptibility. Tobacco cessation efforts have resulted in a drop in oral cancer rates associated with this habit [9], leading to a growing interest in the increased proportion of cases occurring among NS [10]. The increasing incidence of oral cancer in NS petition a better understanding of the natural history of OPML in NS as compared to that of smokers.

OPML with a histological diagnosis of oral epithelial dysplasia (OED) are at an increased risk of progressing to oral cancer than those without dysplasia [11–13]. Although the presence of dysplasia provides an indication of risk for higher grades of dysplasia [14,15], it is a relatively poor predictor for OED with low-grade (mild/moderate) dysplasia, which represent the majority [16]. A more precise risk stratification is required for low-grade lesions.

The study of OPMLs has been the focus of our research team for more than 2 decades, mainly with respect to the development of markers that would help in differentiating progressing from non-progressing mild/moderate dysplasia. The markers included clinical visual aids, such as toluidine blue (TB) staining [17], fluorescent visualization (FV) [18,19] and microsatellite analysis of loss of heterozygosity (LOH) [5].

Microsatellite analysis for loss of heterozygosity (LOH) analysis is used to assess the loss of chromosomal regions that contain known or putative tumour suppressor genes. The Oral Cancer Prediction Longitudinal (OCPL) study being conducted at the BC Cancer Agency in

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Vancouver (British Columbia, Canada) has reported a risk prediction model which uses LOH at key chromosomal loci to stratify lesions to risk of malignant progression [5]. To date, this PCR-based assay is the only marker that has been shown to predict malignant progression of low-grade OED and has been prospectively validated in an independent cohort of patients from community settings [5,20]. Furthermore, it has been optimized for work with archival tissue and small DNA quantities [21–24].

Several studies have examined clinical characteristics and the prognosis of OSCC in NS. However, this question has not been explored thoroughly with respect to OED [25–30]. Not only is the natural history of OED in NS poorly understood, but the path to prevention and intervention of disease is not well defined in this group. There is a gap in the knowledge surrounding the clinicopathological and genetic characterization and the risk of progression in this growing category. This information is critical to the evolution of precision medicine in this subgroup by allowing for medical decisions, practices, and interventions to be tailored to the individual patient based on their predicted risk of disease.

This study reports on findings within the ongoing OCPL study, of which the overall goal is to establish a risk model for the malignant progression of low-grade OED. The purpose of the present study was to characterize the clinicopathological features and the genetic profile of low-grade OED in NS, as well as to compare progression rates and time to progression between NS and smokers with OED. By describing the clinical characteristics of OED in NS, we seek to better define this unique subset of patients and ultimately aid in the prevention, diagnosis, and management of this disease.

Materials and methods

Since January 1, 1997, the OCPL study has prospectively enrolled and followed patients with low-grade OED to a primary endpoint of malignant progression to severe dysplasia, carcinoma *in situ* (*CIS*), or SCC. Participants in the study were identified through a centralized population-based biopsy service, the BC Oral Biopsy Service, where community dentists and specialists across British Columbia (population 4.6 million, in 2014) send biopsies for histological diagnosis. Patients with a diagnosis of low-grade OED were referred by these community clinicians, upon recommendation from the OBS, for follow up to Oral Dysplasia Clinics, where they were invited to participate in the OCPL study. Study protocol and ethical approval was obtained from the University of British Columbia/ BC Cancer Agency Research Ethics Board, and participants were accrued to the study using written informed consent.

The current study is a focused analysis which used a subgroup of the OCPL study population. Eligibility criteria for this analysis required a histologically confirmed primary mild or moderate OED with lesion clinicopathogical and tobacco history available and no prior history of oral cancer. Participants where followed a minimum of 12 months, or to progression, whichever occurred first. No participants were excluded, unless they did not meet the criteria. A total of 445 subjects met the selection criteria and were included in the present analysis, with a median follow-up time of 55.4 months (3.3–241.4 months). Of the 445 cases reported, 269 were reported in a previously published study involving patients with primary OPML [5].

Detailed past and present tobacco and alcohol habits were collected by a standardized questionnaire at study entry. Past and current smoking status, as well as amount and form of tobacco (cigarette, pipe, cigar or smokeless tobacco), were documented. Pipe, cigar and smokeless tobacco were recorded if the subject indicated that they had used this form of tobacco more than once per week for one year or longer [9]. Cigarette equivalents were calculated as one pipe equaled 3 cigarettes, and one cigar equaled 2 cigarettes. Smoker was defined as having consumed more than 100 cigarettes (or the equivalent) in one's life time [31]. Periods of time where a subject had temporarily or permanently quit smoking were recorded. Lifetime smoking history over the subject's entire life, including amount smoked per day during specific age categories, was collated as a pack-year calculation. A pack-year was defined as the equivalent of smoking 20 cigarettes (1 pack) per day for 1 year. Average weekly alcohol consumption was recorded. One alcoholic drink was defined as 8 oz of beer, 4 oz of wine or 1 oz of spirits. Heavy drinker was defined as consumption of more than 14 drinks per week for women and 21 drinks per week for men [32,33].

Clinicopathological data, including lesion site, size, appearance, lesion margin characteristics, as well as information on FV retention and TB positivity were included in the analysis. Lesion size was measured using a calibrated probe and recorded with a bidirectional measurement in millimeters. Lesion appearance was documented as either homogenous (same colour and texture throughout) or as non-homogenous (colour and texture not uniform). Lesion margins were either ill-defined or well-defined. Index lesions were assessed for FV and TB status as previously described [17,19]. LOH analysis was performed on index biopsies collected at baseline, and lesions were classified as low, intermediate or high risk of progression, using previously published methods [5,34].

Clinical follow-up visits occurred every 6 months. Comparative biopsies of the index site were performed upon significant clinical change or approximately every 24 months if no significant change. Outcome was histologically proven progression to severe dysplasia, *CIS*, or SCC. Inclusion of severe dysplasia as the progression endpoint was based on our findings that without treatment, progression occurred in 32% of patients in 3 years; 60% in 5 years [15].

Data analyses were carried out using SPSS® Version 24.0 software (Armonk, NY: IBM Corp). The threshold for significance was set at $P < .05$, and all tests were 2-tailed. The inferential analysis included separate bivariate analyses between each independent and dependent variable. Categorical variables were tested using the Chi-square Test or Fisher's Exact Test when more than 20% of cells contained expected frequencies of < 5. Quantitative variables were tested using an independent samples *T*-test; those that were not normally distributed were tested with the Mann-Whitney *U* test. Interaction effects between tobacco and gender, site and alcohol were evaluated with respect to progression, using a binomial logistic regression model. The main analyses were based on the time-to-event outcome. Time to endpoint was calculated from date of the index biopsy to endpoint date or to last follow-up date (as of Nov 15, 2016), if no progression occurred. Time-to-progression curves and 3-year and 5-year progression rates were estimated using Kaplan–Meier analysis and the Log Rank test. Hazard ratios and the corresponding 95% confidence intervals (95% CI) were determined using the Cox proportional hazards regression model.

Results

Sociodemographic and lifestyle characteristics

A total of 445 subjects were included in the analysis. Approximately one third (31%) of the subjects were NS. Sixty-nine percent of subjects were smokers; 3.4% had reported having used chewing tobacco, 6.5% reported using cigars and 4.9% reported smoking a pipe. Table 1 shows the distribution of cases of OED according to sociodemographic and lifestyle variables in NS as compared to smokers. The majority were Caucasian and over the age of 40, and males were more likely to be smokers than females were. Age at diagnosis was not significantly associated with smoking status. Gender and ethnicity were significant for smoking status ($P = .01$ and $P < .001$, respectively). Alcohol consumption was also associated with smoking status. Heavy consumers of alcohol were 6.6 times more likely to have smoked than those who were light drinkers or who abstained (95% CI, 2.58–16.76; $P < .001$). Gender, ethnicity and alcohol category were each tested in multivariate analysis to see if interaction with smoking status was predictive of malignant progression. When combined with smoking status, neither