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DNA content status using brush biopsy with image cytometry correlated with staging of oral leukoplakia: A preliminary study

Xuan Xiao [a,1], Linjun Shi [a,1], Hongquan Li [a], Yang Song [a], Wei Liu [b,*], Zengtong Zhou [a,*]

[a] Department of Oral Mucosal Diseases, Shanghai Key Laboratory of Stomatology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, China
[b] Department of Oral & Maxillofacial-Head & Neck Oncology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, China

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SUMMARY

Background: Oral leukoplakia (OL) is the best-known potentially malignant disorder of oral cancer. The hypothesis was tested that DNA content abnormality may contribute to risk prediction of malignant potential of OL.
Methods: All OLs were staged according to a clinicopathologic classification and OL-staging system. DNA content status was investigated in a blinded prospective series of OL using brush biopsy with image cytometry, and examined the correlation of DNA content with the clinicopathologic features and OL-staging system in this preliminary study.
Results: Among 65 patients with OL, 27 (41.5%) was identified as DNA content abnormality. The frequency (77.8%) of DNA content abnormality in tongue was higher than that (22.2%) in other oral sites (χ^2 test, $P = 0.038$), and moderate or severe dysplasia had a higher frequency (63.0%) of DNA content abnormality than that (37.0%) of no or mild dysplasia (χ^2 test, $P = 0.022$). Moreover, the odds ratio of DNA content abnormality in high-risk patient group was 5.74-fold (95% confidence interval, 1.81–18.20; $P = 0.003$) increase compared with low-risk patient group. Importantly, the positive correlation between OL-staging system and DNA content status was significant ($P = 0.018$, correlation coefficient = 0.292).
Conclusion: Our findings showed that DNA content status correlated with OL-staging system, suggesting that DNA content abnormality in OL as detected by image cytometry was an early event in oral carcinogenesis. The further large-scale prospective studies with clinical endpoints are warranted to validate the value of DNA image cytometry.

Introduction

Oral cancer is one of the most common malignancies and a leading cause of cancer death worldwide, and oral leukoplakia (OL) is the best-known potentially malignant disorder of oral cancer [1,2]. Early detection and diagnosis of high-risk OL before malignant transformation is of utmost importance for effective intervention and is a high priority for reducing morbidity and mortality due to oral cancer [3]. Currently, tissue harvesting by scalpel biopsy and histological examination based on the presence and the degree of epithelial dysplasia is the gold standard for determining malignant potential risk of OL. However, it is a well-known fact that this histologic classification is insufficient and may involve subjectivity [4]. Besides, it is evident that invasive sequential biopsies have a limited reproducibility for the surveillance of patients with suspicious lesions. Therefore, adjunctive diagnostic techniques are required to identify the high-risk OL before transformation.

Brush biopsy with DNA image cytometry is an objective and noninvasive adjunctive diagnostic technique to automatically measure nuclear DNA content (ploidy) in a fixed cell suspension deposited on a glass slide [5]. DNA aneuploidy (content abnormality) determined by image cytometry is internationally accepted as a marker of malignant transformation of cells, which is the cytometric equivalent of chromosomal aneuploidy [6–8]. DNA content abnormality is thus an important indicator of numerical chromosomal alterations and its emergence is often a critical early event during carcinogenesis [5]. Using the DNA image cytometry, an increase in both sensitivity and specificity of oral brush biopsy up to 100% for early diagnosis of oral cancer has been reported in the previous cross-section studies [9–14]. However, hardly any studies are available so far focusing on examining brush biopies of OL by DNA image cytometry [15].

* Corresponding authors. Tel.: +86 21 23271699; fax: +86 21 63087076.
E-mail addresses: liuweb@hotmail.com (W. Liu), zhouzengtong@hotmail.com (Z. Zhou).
[1] X. Xiao and L. Shi contributed equally to this work.

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We hypothesize that DNA content abnormality may contribute to risk prediction of malignant potential of OL. The purpose of this preliminary study was to investigate the association of DNA content with the clinical and pathologic parameters in a blinded prospective series of OL patients, and to evaluate the association of DNA content abnormality with both high-risk site of tongue and moderate or severe dysplasia as high-risk patient group, based on low- and high-risk categories in a recent relevant study on DNA content using flow cytometry [16].

Materials and methods

Patients and sampling procedure

This study was approved by the institutional review board of Shanghai ninth People's Hospital ([2012]21), and written informed consent was obtained from all patients. In this preliminary study, 65 patients with OL, who visited the clinic at the Department of Oral Mucosal Diseases, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine during March 2013 to April 2014 period, were prospectively enrolled. Clinical data, including age, gender, smoking, alcohol intake, diet habit, and lesion site were recorded.

Before scalpel biopsies were performed of the suspicious OL lesions, every participate underwent a brush biopsy before treatment. Brush samples were collected using the Oral cytobrush kits (CytoSavant, Motic Inc., Xiamen, China) by gently pressing and rotating a flexible cytology brush over 10 times in one direction. The brush head was then placed in a cytology fixative vial and the cells were transferred to slide. Brush samples analysis was performed at the Laboratory of Motic Inc. Scalpel biopsy was then taken from the same location of brush sample, and the biopsy was fixed in formalin, embedded in paraffin, and processed for routine histopathologic examination at the Department of Oral Pathology of our hospital.

Histologic examination

The World Health Organization (WHO) criteria [17,18] for OL and epithelial dysplasia were used when examining the histopathology of the sections. According to the WHO definition of OL [18], "A white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer", the patients with the clinical appearance and histopathologic changes of oral white or predominantly white oral diseases such as leukoedema, linea alba, chronic biting irritation, and while spongy nevus, and other potentially malignant disorders such as discoid lupus erythematosus and lichen planus were excluded in this study. The presence of dysplasia was graded as mild, moderate, or severe, which was blind to the DNA content results. The grade of dysplasia was classified into low-grade (no or mild) dysplasia and high-grade (moderate or severe) dysplasia. This binary system to grade dysplasia proposed by WHO [4,19] was intended to reduce the subjectivity inherent to grading dysplasia. In addition, all lesions were classified as tongue and nontongue sites according to the published literature [19–21]. It is generally accepted that tongue is the high-risk subsite and high-grade dysplasia carry a higher risk for transformation than low-grade dysplasia [19,20]. We thus defined high- and low-risk group as tongue leukoplakia with moderate or severe dysplasia and nontongue leukoplakia with no or mild dysplasia, respectively, based on low- and high-risk categories in a recent relevant study on DNA content using flow cytometry [16]. As shown in supplementary data Table S1, the size, presence and degree of dysplasia were incorporated into the OL-classification and OL-staging system [21].

Measurement of DNA content

Brush samples were spread on microscope slides, and the slide depositions were stained with Feulgen-thionin method. Measurements of DNA contents were performed using an automated DNA image cytometer (MotiSavant, Motic Inc., Xiamen, China), and the data was analyzed using Motic imaging software (MotiClassify, Version No.2.12.3, Motic Inc.). Samples with a minimum of 300 nuclei were selected for analysis, which was blinded against the histological results. Each specimen was normalized by a normal control epithelium nuclei. The DNA index (DI) was the ratio of analyzed nuclear DNA content to the reference DNA content. Based on the criteria for evaluation of the previous reports [14,22,23] and ESACP guidelines [6], the samples were classified as DNA content abnormality if the test results met at least one of the following three conditions: (i) two separate G0–G1 peaks (diploid G0–G1 peak with DI of 0.98–1.02, aneuploid G0–G1 peak with DI of 1.05–1.9 or 2.1–3.8), (ii) more than 10% of the nuclei with DI of 1.25–2.3 and DI $\geqslant$ 2.3, (iii) more than 3 nuclei (1% of a minimum of 300 nuclei) with DI $\geqslant$ 2.3.

Statistics

Data collection was conducted using the Microsoft Office Excel package and processed with the SPSS 16.0 software package (SPSS Inc. Chicago, IL) for the statistical analyses. The Student's *t*-test and χ^2 or Fisher's Exact test was used to assess the differences among quantitative variables and qualitative variables, respectively. The correlation between OL-staging system and DNA content status was determined by Pearson correlation analysis. To evaluate the association of the DNA content versus the low- and high-risk patient groups, logistic regression was applied to evaluate risk ratios and the association among variables, and odds ratios (OR) and their 95% confidence intervals (CI) were calculated. All the tests were two-sided, and *P* values <0.05 were considered statistically significant.

Results

Association of DNA content with the clinicopathologic features of OL

Among 65 patients with OL who prospectively enrolled in this study, a total of 27 (41.5%) patients was identified as DNA content abnormality. Representative clinical manifestation, histopathology and DNA content analysis of a same patient is shown in Fig. 1. The association of DNA content with the clinicopathologic features of OL is presented Table 1. The frequency (77.8%) of DNA content abnormality in tongue was higher than that (22.2%) in other oral sites (χ^2 test, $P = 0.038$), and moderate or severe dysplasia had a higher frequency (63.0%) of DNA content abnormality than that (37.0%) of no or mild dysplasia (χ^2 test, $P = 0.022$). The differences of DNA content status in age, gender, diet habit, smoking, alcohol intake were not observed.

Odds ratio analysis of DNA content abnormality in OL

Table 2 reports the OR analysis by logistic regression model for assessing the association between DNA content abnormality in OL and the clinicopathologic features and the low- and high-risk patient groups. Univariate analysis revealed that gender, diet habit, smoking, and alcohol intake were not significantly associated with DNA content, whereas tongue site (OR, 3.50; 95% CI, 1.16–10.60; $P = 0.027$) and dysplasia (OR, 3.68; 95% CI, 1.30–10.40; $P = 0.014$) were significantly associated with DNA content. To further assess the influence of each factor, we did a multivariate analysis. Tongue