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HEAD AND NECK

Brush biopsy with DNA-image cytometry: a useful and noninvasive method for monitoring malignant transformation of potentially malignant oral disorders

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Abstract Oral and pharyngeal cancer is the sixth most common cancer worldwide, the 5-year survival rate has not yet increased. A key factor in rates not having improved is the lack of early detection. This study was undertaken to estimate the diagnostic accuracy of brush biopsy with DNA-image cytometry (a noninvasive method) for potentially malignant oral disorders compared with tissue biopsy pathology in China. Exfoliative cells were obtained using a cytobrush cell collector from oral mucosa of 52 subjects, followed by scalpel biopsy from the same region. Nuclear DNA contents (ploidy) were measured after Feulgen restaining, using an automated DNA image cytometer. Exfoliative cytology with DNA-image cytometry and histopathological diagnosis were performed separately at different institutions. Histological investigation was considered the gold standard. We reported that the sensitivity of DNA aneuploidy for the detection of cancer cells in potentially malignant oral disorders was 86.36 %, its specificity was 90.00 %, its positive predictive value was 86.36 %, and its negative predictive value was 90.00 %. Brush biopsy with DNA-image cytometry is a useful method for monitoring potentially malignant oral disorders.

Keywords Brush biopsy · Oral exfoliative cells · DNA aneuploidy · Potentially malignant oral disorder · Non-invasive screening method

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Introduction

Oral and pharyngeal cancer is the sixth most common cancer worldwide. The annual estimated global incidence is approximately 2,75,000 for oral cancers. Parts of Asia (e.g., China, India and Pakistan) are areas characterized by high rates of oral cancer [1]. Despite numerous surgical, chemotherapeutic and radiotherapeutic remedies, the 5-year survival rate has not yet increased, and the rate is not significantly better for patients who have survived advanced oral cancer, as they suffer imperfections in their appearances and in their oral functioning. A key factor in rates not having improved is the lack of early detection and the consequent failure of valid early treatments for oral cancer [2–6].

The term 'potentially malignant disorders' indicates that there is a family of morphological alterations, among which some might have increased potential for malignant transformation [7]. Oral potentially malignant epithelial lesions (PMELs), including oral lichen planus (OLP),

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leukoplakia, erythroplakia, palatal lesions in former smokers, oral submucous fibrosis, actinic keratosis and discoid lupus erythematosus, are common oral mucosa diseases, and these lesions can involve dysplasia or carcinoma in situ despite being clinically 'normal' appearing [7]. At the same time, it is difficult to achieve early diagnosis of oral cancer due to the uncertainty of visual oral examination.

The only accepted method to identify suspicious oral lesions is scalpel biopsy with histopathological diagnosis, but many patients hesitate to accept scalpel biopsy because the method is invasive, especially when the lesions appear in seemingly 'normal' oral mucosa.

Today, a non-invasive and easily practicable method, DNA aneuploidy, has been introduced for the early diagnosis of malignant transformation of squamous epithelial cells. DNA-aneuploidy, which is the cytometric equivalent of chromosomal aneuploidy, is widely believed to be a marker of neoplastic cell transformation [8–10]. Brush biopsy with DNA-image cytometry, an adjuvant to the cytological diagnosis of oral mucosal smears, is also an objective method for detecting DNA aneuploidy [11–13]. However, there have not been many reports in China on the accuracy of brush biopsy with DNA-image cytometry in diagnosing malignant transformation of potentially malignant oral disorders. In the majority of current studies, scalpel biopsy was performed after brush biopsy of lesions with high-risk clinical features but not after brush biopsy of innocuous-looking lesions [14]. The aim of our study was to estimate the diagnostic ability of EC combined with DNA-image cytometry applied in patients with potentially malignant oral disorders.

Materials and methods

Patient population

This study included 52 subjects who sought examination and remedy of oral lesions at the Department of Oral Medicine, Stomatological Hospital of Jiangsu Province (Nanjing, China), from February 2012 to November 2012. Informed consent was acquired from the patients, and all of the procedures were approved by the Ethical Committee of Stomatological Hospital Affiliated to Nanjing Medical University. Features of the report population are summarized in Table 1.

Table 1 Subjects and oral mucosa characteristics

Variable	Number
Gender	
Female	37
Male	15
Age (years)	
Average	58
Minimum	19
Maximum	84
Lesion types	
OLP	21
Leukoplakia	6
Palatal lesions in reverse smokers	4
Gingivitis desquamativa	6
Discoid lupus erythematosus	2
Tone ulcerous lesions	4
Neoplasm	4
Normal	5

OLP oral lichen planus

Clinical procedure

Before biopsies were performed of the suspicious lesions, every subject underwent a brush biopsy. To obtain smears, we used a cytobrush cell collector, which was rolled at the location of the mucosal lesion at least three times using gentle pressure. The exfoliative cells were transferred to two slides, which were immediately fixed with absolute ethanol. Nuclear DNA contents (ploidy) were measured after Feulgen staining using an automated DNA image cytometer [10, 15, 16]. The smears and biopsy tissues were examined at different institutions.

Results evaluation and analysis

Every normal cell has a fixed DNA content, so DNA index (DI) or c (content) was used as a unit. When the cells were in the G1/G0 phase, cellular DNA content was set to DI = 1 or 2c. When the cells were proliferating, the DNA content doubled, and DI = 2 or 4c. A lesion was classified as DNA diploid if there was only one DNA stemline (STL) between 1.80 and 2.20c. A lesion was characterized as DNA polyploid if there were DNA-STLs between 1.80 and 2.20c and between 3.60 and 4.40c. DNA aneuploidy was assumed if there were abnormal STLs <1.80c and >2.20c or <3.60c and >4.40c and/or 5c exceeding events (5cEEs) >0 [17, 18].

DNA ploidy measurements (DNA diploid or DNA aneuploid) were compared with the gold standard of histopathological diagnosis. Negative diagnoses of DNA cytometry indicated DNA euploid (DNA diploid and DNA polyploid), whereas positive diagnoses indicated DNA-aneuploid histograms. Histopathologically negative results indicated non-neoplastic, mild and moderate dysplastic cells, whereas histopathologically positive results indicated severely dysplastic or malignant cells.

We compared the results between DNA cytometry and histopathological diagnosis. True-positives of the DNA-cytometric indicated that the cases presented as DNA aneuploid, and the pathological diagnosis was severe dysplasia or neoplasia. False-positives presented as DNA aneuploid, and the pathological diagnosis was normal or mild dysplasia. True-negatives presented as DNA euploid, and the pathological diagnosis was normal or mild dysplasia. False-negatives presented as DNA euploid, and the pathological diagnosis was severe dysplasia or neoplasia. The sensitivity and specificity were calculated according to a four-field table (Table 2):

Sensitivity	True-Positives/(True-Positives + False-Negatives) × 100 %;
Specificity	True-Negatives/(True-Negatives + False-Positives) × 100 %;
Positive predictive value	True-Positives/(True-Positives + False-Positives) × 100 %;
Negative predictive value	True-Negatives /(True-Negatives + False-Negatives) × 100 %;
False positive rate	False-Positives/(True-Positives + False- Positives) × 100 %; and
False negative rate	False-Negatives/(True-Negatives + False-Negatives) × 100 %

Table 2 Comparison of results between DNA-image cytometry and pathological diagnosis

Pathological diagnosis	DNA-image cytometry	
	Positive (cases)	Negative (cases)
Positive (cases)	19	3
Negative (cases)	3	27

Results

We compared every subject's diagnosis of DNA-image cytometry (e.g. Figs. 1, 2) with the gold standard of histopathological diagnosis (e.g. Fig. 3). Table 2 shows the comparison of results between DNA-image cytometry and pathological diagnosis in 52 subjects. Of these, 22 patients exhibited malignant epithelial lesions and 30 patients had no malignant epithelial lesions according to pathological diagnoses. In the 22 malignant epithelial lesions, DNA-image cytometry revealed 19 subjects with the presence of DNA aneuploidy. In the 30 normal cases, DNA-image cytometry revealed 27 subjects without presence of DNA aneuploidy. The sensitivity of DNA-image cytometry was 86.36 %, its specificity was 90 %, its positive predictive value was 86.36 %, and its negative predictive value was 90 %. The false positive rate was 13.64 %, and the false negative rate was 10 %.

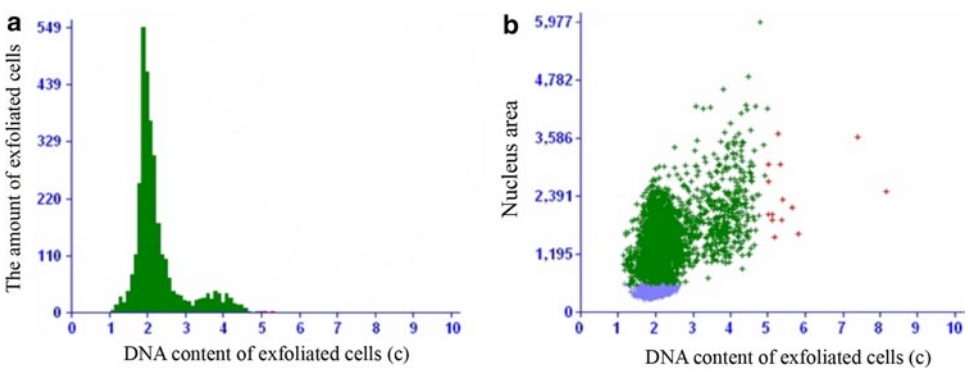

Fig. 1 Several cells DNA content were more than 5c (**a, b**). Brush biopsy with DNA-image cytometry, exfoliated cells taken from the right ventral tongue of a 77-year-old female subject

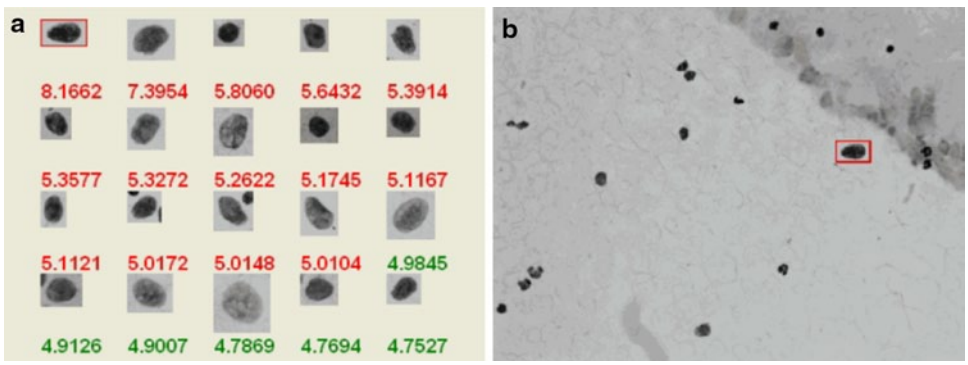

Fig. 2 Several cells DNA content were more than 5c (**a**). The cell which had the most DNA content was showed (**b**). Brush biopsy with DNA-image cytometry, exfoliated cells taken from the right ventral tongue of that 77-year-old female subject

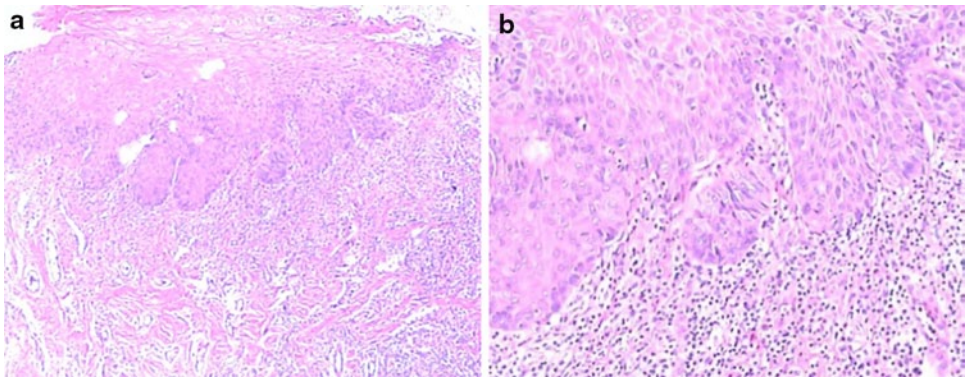

Fig. 3 Mucosal epithelial moderate to severe dysplasia (HE). Histopathology of biopsies, taken from the right ventral tongue of that 77-year-old female subject

Discussion

The development of oral squamous cell carcinoma (OSCC) is a continuous process, including atypical hyperplasia, carcinoma in situ and infiltrating carcinoma. Deepak Kademani [19] highlighted the importance of histologic grade and the TNM staging system as independent factors in predicting survival in patients with OSCC. They found that the mean 5-year survival rate, with a 95 % confidence interval for grade 1, ranged from 54 to 80 %; grade 2, from 41 to 62 %; and grade 3, from 29 to 70 %. There was an average decrease in patient survival of 44 % per grade of tumor [19]. Therefore, researchers are seeking a method that is sensitive, specific, more compliance-inspiring and easily practicable, to detect malignant transformation of the oral epithelium. In our study, brush biopsy with DNA-image cytometry showed high sensitivity (86.36 %) and specificity (90 %) in detecting malignant transformation of the oral epithelium. Potentially malignant oral disorders have increased potential for malignant transformation [7]. Considering the painless and noninvasive procedure to obtain smears and the high sensitivity and specificity of brush biopsy with DNA-image cytometry in our study, we believe that brush biopsy is easily practicable for monitoring of oral disorders. Compared to the standard diagnostic method of 'scalpel biopsy', brush biopsy resulted in high compliance. In light of the above, the brush biopsy with DNA-image cytometry method is useful to monitor potentially malignant oral disorders.

Vital staining, such as toluidine blue (TB) solution, a vital dye that is believed to stain nucleic acids, has been used in the detection of OSCC for many years. Analysis of the current evidence suggests that TB is good at detecting carcinoma, but its sensitivity in detecting dysplasia is significantly lower [21, 22]. It is worth mentioning that there remain a large proportion of false-positive stains. The sensitivity of vital staining was approximately 84–97.3 %, its specificity was 41.2 %, and the false positive rate was 42.2 %, which negatively affects its use in primary care settings as a valid screening method for detecting malignant transformation [23].

Additionally, we should note that we reported the false positive and negative cases in our study: the false positive rate was 13.64 %, and the false negative rate was 10 %. Previous studies reported that DNA-cytometric diagnosis of malignancy approximately 1–15 months before histological diagnosis [11, 20] could explain, to some extent, why the false positive diagnosis occurred. Exfoliative cells overlapping on smears can also lead to false positives. Inflammatory exudate and necrotic chipping can impact procedures to obtain exfoliative cells, contributing to the false negative rate. Therefore, we should avoid obtaining exfoliative cells during this stage of disease. Preliminary treatment of potentially malignant oral disorders, when they present inflammatory exudate or necrotic chipping, is good for both brush biopsy and scalpel biopsy. We must attempt to reduce the false positive and false negative rates in the following ways: (a) there should be good distribution of exfoliative cells on smears; and (b) examinations should be performed when the exudate and crust have subsided.

DNA aneuploidy is internationally accepted as a marker of neoplastic cell transformation, which is the cytometric equivalent of chromosomal aneuploidy [9, 10]. Using DNA-image cytometry, we were able to judge the condition of potentially malignant oral disorders and whether they underwent malignant transformation [24, 25]. Furthermore, these tests can contribute to the early detection of oral cancer and early treatment. Thus, brush biopsy with DNA-image cytometry is suitable for monitoring suspicious oral lesions when repeated asynchronous examinations are necessary during the clinical course because it is noninvasive and easy to accept by patients.

Based on the above findings, brush exfoliative cells with DNA-image cytometry of all visible oral lesions, if they are considered clinically suspicious for cancer, is an easily practicable, non-invasive, painless screening method for the detection of precancerous oral lesions. We conclude that DNA-image cytometry is a useful adjuvant tool for the monitoring of neoplastic epithelial cells in potentially malignant oral disorders, and it is suitable for monitoring suspicious oral lesions when repeated asynchronous

examinations are necessary during the clinical therapic course. Scalpel-biopsy can contribute to the final diagnosis, but it is difficult to smoothly carry out in monitoring whether potentially malignant oral disorders turned malignant. Oral brush biopsy with DNA-image cytometry probably will not substitute histopathology in the decisive diagnosis of oral cancer, but it probably is meritorious for the screening of lesions that would be likely to develop to oral cancer.

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Ethical approval The study was approved by the Ethical Committee of Stomatological Hospital Affiliated to Nanjing Medical University (No. PJ2011-012-03). The authors declare that our studies have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and that all persons gave their informed consent prior to their inclusion in the study.

Conflict of interest The authors declare that they have no conflicts of interests.

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