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Case Report

Non/micro-invasive clinicopathologic methods in the assessment of oral leukoplakia multistep carcinogenesis: a case report

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Abstract: Oral leukoplakia is a common potentially malignant oral lesion. Most scholars consider that its cancerization follows a multistep process of gradual progression. If surveillance and assessment could be performed at earlier stages to assess the risk of cancerization, the probability of malignancy would obviously be reduced. In this paper, we report on a patient with cancerous oral leukoplakia in three different clinical areas, from upper to lower, that showed a similar spatial distribution of increased cancer risk in each clinical manifestation. A series of non- or microinvasive clinicopathologic methods and subsequent histopathologic examination were performed and proliferation of related proteins was investigated by immunohistochemistry. The areas with different levels of cancer risk coexisted successively and contiguously in one lesion. This case intuitively confirmed the oral leukoplakia multi-step carcinogenesis model, non-invasive clinicopathology, histopathology and immunohistochemistry, showed a positive role for noninvasive detective methods in oral leukoplakia long-term monitoring management.

Keywords: Oral leukoplakia, carcinogenesis, toluidine blue staining, visual enhanced lesion scope, oral brush biopsy

Introduction

Oral leukoplakia (OLK) is a common oral potentially malignant lesion (OPML) which is considered to have increased risk of transformation into oral cancer [1]. The reported overall malignant transformation rate is approximately 0.13% to 17.5% [2-4]. Carcinogenesis of OLK is usually a multistep and seemingly uneventful long term process, the period of malignant transformation varies from less than 5 years to 25 years, depending on the level of dysplasia [2]. Therefore, a safe and harmless technique to monitor OLK is important for clinicians. Toluidine blue (TB) staining, visually enhanced lesion scope (VELscope) examination and oral brush biopsy are useful methods to monitor and assess the progression of carcinogenesis in real time, which helps take preventative measures against the development of oral squamous cell carcinoma (OSCC) [5].

Recently, we treated a patient with left ventral tongue OLK, the lesion appeared in three different clinical areas (white plaque, granular proliferation and atrophy erosion) from upper to lower. We examined the lesion with series of non-/micro invasive clinicopathologic methods, the results showed that the cancer risk level gradually increased from upper area to lower areas. The subsequent histopathologic examination and immunohistochemistry of proliferation related proteins showed the lesion possessed typical multi-step carcinogenesis characteristics that the normal epithelium, hyperplasia, mild, moderate, and severe dysplasia (carcinoma in situ), and local invasive carcinoma coexisted successively and contiguously

in one lesion. This case intuitively confirmed the oral leukoplakia multi-step carcinogenesis model in clinical manifestations, non-invasive clinicopathology, histopathology and immunohistochemistry and showed positive role of noninvasive detective methods in oral leukoplakia long-term monitoring management.

Case presentation

A 37-year-old woman of Han nationality, was first admitted on 29th March 2012 in Sichuan University Stomatological Hospital. She complained of a white plaque on the left ventral surface of her tongue, with a sensation of roughness for one year, and increased dull pain (NRS=4) for two months. She had taken some vitamins and self-medicated using a compound chlorhexidine gargle, with no obvious effect over approximately two weeks. She denied having tobacco or alcohol habits or a family history of cancer. She had not received any other treatment before admission to the hospital. She reported no other health problem or complication. There was no diet risk and no systematic symptom typical of Candida infection.

Oral examination: Normal tongue activity. Red and white lesions over the left ventral surface of the tongue extending to the mucosa of the mouth floor. The upper area consisted predominantly of slightly raised white keratotic plaque and patches; the mid area was dominated by mixed red and white lesions with granular hyperplasia, which were obviously raised and rough on their surfaces; the lower area was atrophic and eroded with some pseudomembrane, a concave center and raised margins, and was firm when touched, but without basement infiltration (**Figure 1A**). No enlarged submaxillary or cervical lymph nodes were palpable. Teeth and periodontal condition were good, and neither questionable restoration nor local stimulus was found.

Upon getting informed consent from the patient, we examined the lesion with non-/micro invasive clinical pathology methods, firstly TB staining, then VELscope examination (LED Dental, Vancouver, BC, Canada), and oral brush biopsy (OralCDx Laboratories, Suffern, NY). These were all performed at the first hospital visit.

Toluidine blue (Shanghai Ruji Biology Technology Ltd, Shanghai, China) staining showed that there were no obvious colored points in the upper area, a small number of sporadic light-blue points in the mid area, and many dark-blue points in the lower area (**Figure 1B**).

VELscope examination presented no obvious fluorescence loss in the upper area. Sporadic areas of fluorescence loss could be observed not only in the mid area, but also in the benign-looking mucosa distal from the mid area, while the whole lower area showed fluorescence loss (**Figure 1C**). Comparing the scope of fluorescence loss (yellow dotted line) with the range of the lesion (red dotted line), we found that the area of fluorescence loss was mainly located within the lesion; however, fluorescence loss also occurred in the normal-appearing mucosa distal and adjacent to the lesion. The scope of surgical excision encompassed the range of both the visible lesions and the area of fluorescence loss (**Figure 1D**).

The procedure for oral brush biopsy was performed according to the instructions. The cell specimen collected by the brush was transferred to a slide and fixed with fixative containing 95% ethyl alcohol and 2.5% carbowax for 15 mins. The slide was then stained with Papanicolaou stain, and observed by the pathologists. The results showed that normal keratinocytes with small nuclei and light staining were seen in the upper area, with no observable epithelial abnormality. Abnormal epithelial cell changes occurred in the mid area, including the presence of larger cell nuclei in individual cells. Unlike the areas mentioned above, the cell nuclei in the lower area were larger and obviously hyperchromatic (**Figure 1E-G**).

All these examinations indicated that it was a high risk lesion and the patient was advised to have the lesion dissected.

The patient was admitted to the oral oncology ward. Examination showed no abnormal results for routine blood examination, blood glucose, coagulation function, and blood biochemistry. Hepatitis B testing showed that the patient was positive for antibodies to hepatitis B, E antigen and hepatitis B core antigen. The chest X-ray was normal; drug allergy testing showed that the patient was allergic to cefuroxime sodium, mezlocillin sodium, and sulbactam sodium. Then extensive resection of the lesion and tissue patch reparation surgery was performed. There was no evidence of infiltration at the edge of the lesion and the lymph nodes were not dissected.

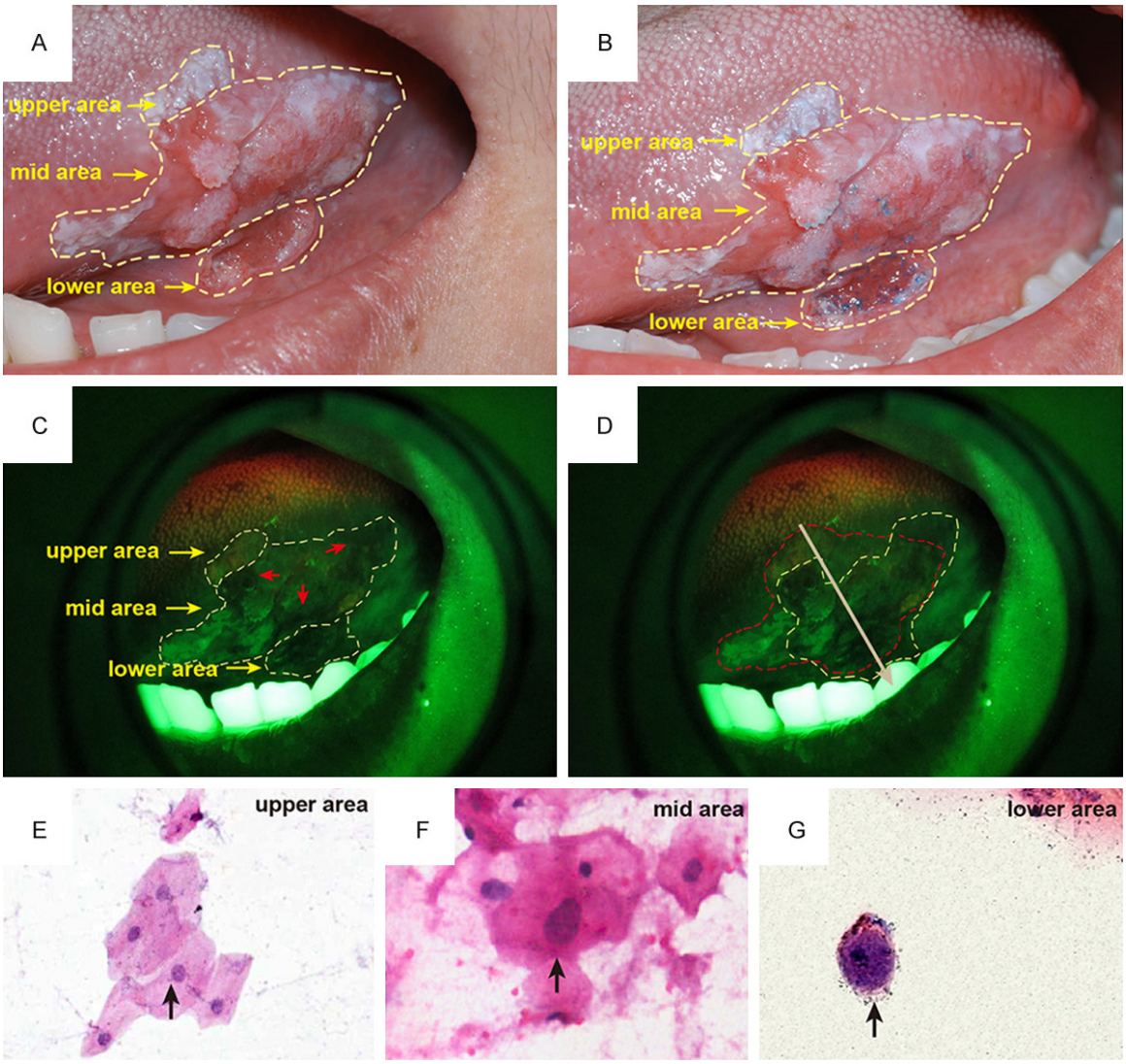

Figure 1. The Partition of the Left Ventral Tongue Lesion and Results of Noninvasive Detection Methods. (A) The lesion on the left ventral surface of the tongue. (B) The result of toluidine blue staining. (C) The result of autofluorescence detection. (D) The scope of fluorescence loss (yellow dotted line) was beyond the range of the lesion (red dotted line). (E) The oral brush biopsy of the upper area mainly contained cells with small nuclei and light staining (black arrow). (F) Larger nuclei were observed in individual cells in the mid area (black arrow). (G) Larger cell nuclei were observed in the lower area (black arrow). The oral brush biopsy images (E-G) are magnified *400.

After being embedded in paraffin, the surgery specimen was sliced along the longitudinal axis (**Figure 1D**, white arrow) and then stained with hematoxylin and eosin. The pathological results showed that it was a local superficial invasive squamous cell carcinoma: normal epithelium, epithelial hyperplasia without dysplasia, mild dysplasia, moderate dysplasia, severe dysplasia (carcinoma in situ), and local invasive carcinoma coexisted in the lesion (**Figure 2A-G**).

Immunohistochemistry tests were performed on the surgery specimen to examine the expression of proliferating cell nuclear antigen (PCNA) and wild-type p53 using monoclonal antibodies specific for PCNA (1:25 dilution, ZM-0213; ZSGB-BIO, China) or wild-type p53 (1:50 dilution, ZA-0501; ZSGB-BIO, China) according to the manufacturer's instructions for the Histostain-SP kits (SP-9000; ZSGB-BIO, China). In normal and hyperplastic epithelium, PCNA was expressed at a low level, and PCNA-positive cells were mainly present in the basal layer (**Figure 2I**, **2J**). In mild and moderate dysplasia, the number of PCNA-positive cells increased in the basal and suprabasal layers (**Figure 2K**,

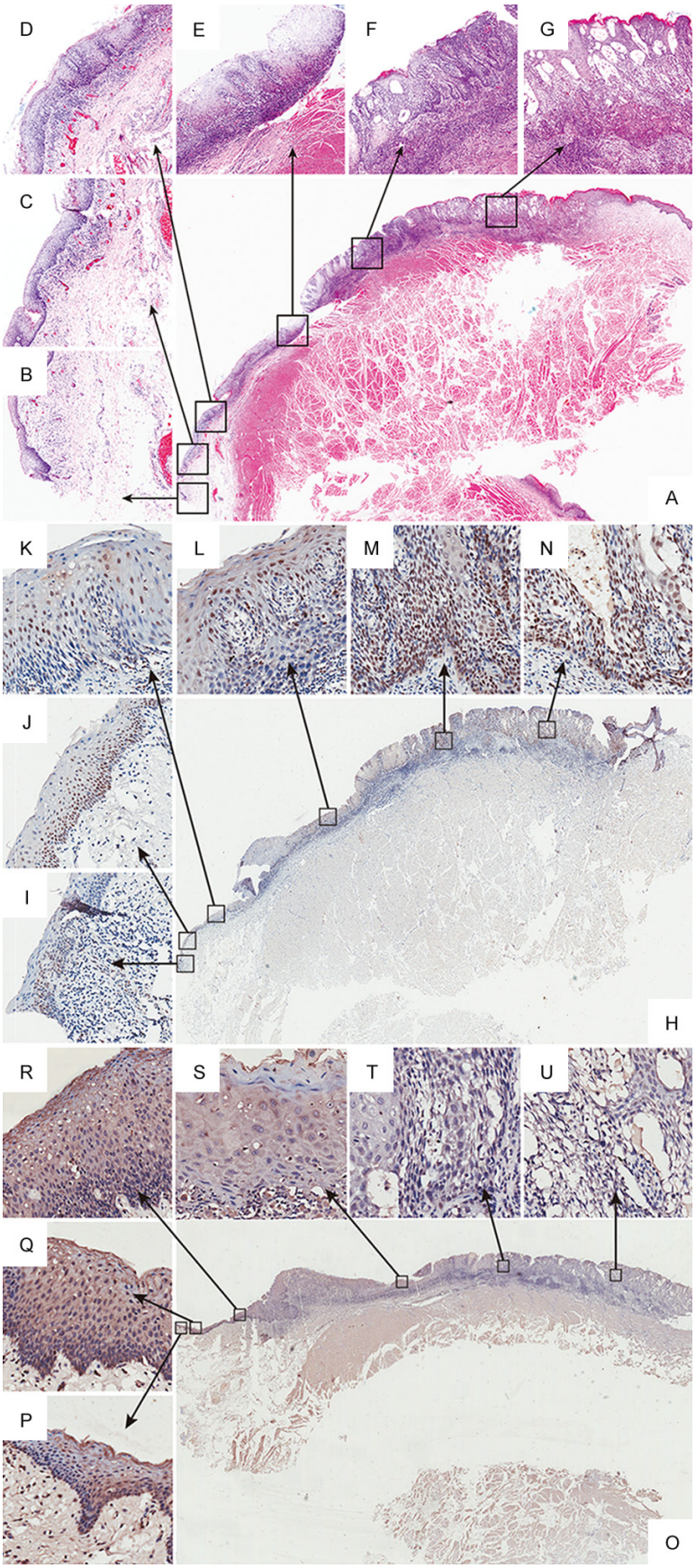

Figure 2. The Pathological and Immunohistochemical Manifestations of the Left Ventral Tongue Lesion. A. The pathological picture of the whole lesion (×10). B. Normal epithelium (×50). C. Epithelial hyperplasia (×50). D. Epithelial mild dysplasia (×50). E. Epithelial moderate dysplasia (×50). F. Epithelial severe dysplasia/carcinoma in situ (×50). G. Local invasive carcinoma (×50). H. PCNA expression in the lesion (×10). I, J. PCNA-positive cells mainly located in the basal layer of normal and hyperplastic epithelium (×200). K, L. PCNA-positive cells increased in basal and suprabasal layers in mild dysplasia and moderate dysplasia (×200). M, N. PCNA obviously increased in the whole epithelial layer of severe dysplasia/carcinoma in situ and invasive carcinoma (×200). O. Wild-type P53 expression in the lesion (×10). P53 expression decreased from normal mucosa to invasive carcinoma. P-R. Wild-type p53 mainly expressed in nucleus and cytoplasm of normal, hyperplastic epithelium and in mild dysplasia (×200). S-U. P53 expression clearly decreased in severe dysplasia/carcinoma in situ (×200).

2L). In severe dysplasia (carcinoma in situ) and invasive carcinoma, the expression of PCNA clearly increased in the whole epithelium (**Figure 2M**, **2N**). Wild-type p53 was mainly expressed in the nucleus and cytoplasm of normal and hyperplastic epithelium and in mild dysplasia (**Figure 2P**, **2Q**). In moderate dysplasia to invasive carcinoma, the expression of p53 clearly decreased (**Figure 2R-U**).

Five days later, the patient was discharged from hospital. To date, the patient has been followed up once every 2-3 months for 18 months. The last follow-up was 17th December 2013. The operation wound had healed well with a soft texture; the tough

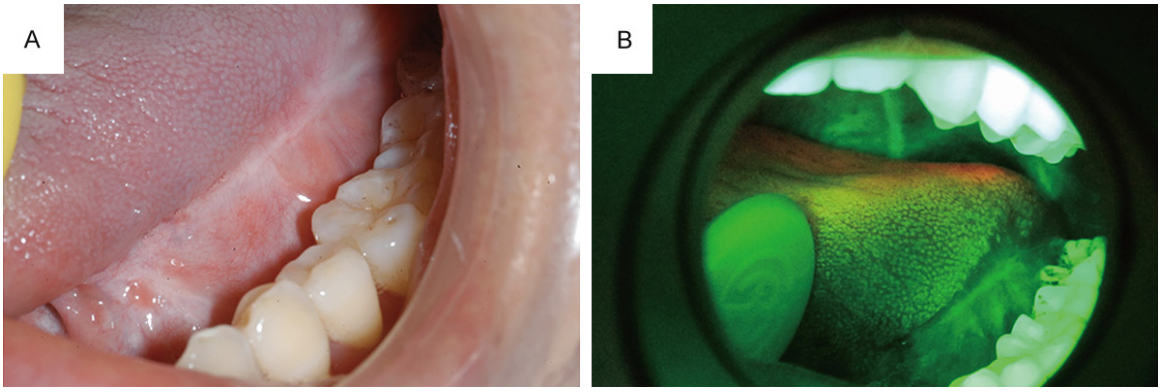

Figure 3. Eighteen months after expanded resection of left tongue lesion. A. The wound healed well and the function of the tongue recovered. The toluidine blue staining was negative. B. Autofluorescence evaluation was normal.

moves were good, and there were no obvious abnormalities regarding swallowing or voice. There was no pain or edema (NRS=0) around the wound, no ulcer or tissue proliferation. TB staining and VELscope were performed for each follow-up, and all the results were negative. Her condition has remained stable without recurrence (**Figure 3A**, **3B**).

Discussion

The likelihood of transformation in OLK is thought to be dependent upon the histology of the lesion (homogeneous versus non-homogeneous), clinical subtype, large size, oral location, previously diagnosed cancer in the head and neck region, older age, female gender, absence of smoking habits and duration of the leukoplakia [6]. In this case, the leukoplakia was located on the ventral surface of the tongue and the floor of the mouth, one of the most common sites for OSCC, and it also had heterogeneous characteristics including white keratinization, granular hyperplasia, and atrophic erosion, which suggested that it was a type of lesion that had a high risk of canceriza-tion [7]. We investigated and analyzed the lesion with three non-/micro-invasive detection methods to forecast the risk of cancerization and the invasive range, and to explore whether the trend of malignant grade variation found by these methods was consistent with the clinical manifestations.

Toluidine blue is a dye that can combine specifically with nucleic acids and abnormal tissue, and the presence of stain is correlated with tissue dysplasia, and it is easy to perform and with high specificity [8, 9]. In this case, the white patch in the upper area of the lesion was not stained at all, suggesting that it was a relatively benign area without active proliferation. The degree of staining increased gradually in the mid and lower areas, suggesting that these two areas were more active than the upper area, and that dysplasia or even carcinoma was present.

VELscope detection is a useful method to diagnose early oral cancer and define the margin for surgery [10, 11]. The autofluorescence is decreased in abnormal mucosa, which appears dark, while normal tissue is green in color. VELscope has high sensitivity, however, local inflammation, small vessels, and melanin in the oral cavity may disturb detection and give a false positive result [12, 13]. In this case, there were no obvious interfering factors. The loss of fluorescence was mainly limited to the mid and lower areas, while the range of fluorescence loss was beyond the visual margin of the lesion, providing an important reference to determine the surgical margin alongside oral examination.

Oral brush biopsy identifies tissue properties through the lesion's cytology and has overall high accuracy; however, it is easily influenced by the quality of specimen [14, 15]. We collected cell specimens from the three areas of lesion. The results indicated an increasing trend towards malignancy from upper area to lower area.

The use of these three methods in combination might provide complementary advantages. TB staining was used to increase specificity of examination, VELscope for sensitivity, and oral

brush biopsy for accuracy; meanwhile, VELscope could be used for determination of the range of the lesion. These were sequential examinations, from non-invasive to minimized invasive examination from several aspects. [16]. After comprehensively analyzing the results obtained by these methods, we concluded that the lesion was transiting from OLK into OSCC, and this tendency was confirmed by the subsequent histopathologic and immunohistochemical tests. In addition, wild-type p53 expression decreased and expression of PCNA increased gradually from normal tissue to the invasive carcinoma parts of the lesion.

OPML is a disease that requires long-term management including the definition of tissue properties, decisions about the location and time for biopsy or surgery, and long-term surveillance after surgery. In this case, we used a combination of three methods to classify the tendency to malignancy and designed a suitable therapeutic schedule. After surgery, 18 months of continuous follow-up was performed with regular noninvasive surveillance, and there was no recurrence. This showed that the combined use of these methods could be appropriate for long-term surveillance of OPMLs and to effectively prevent the progression of carcinogenesis.

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Disclosure of conflict of interest

None.

Authors' contribution

Hongmei Zhou, Mei Lin designed and guide the studies. Changlei Wei, Qinghong Gao carried out the studies. Changlei Wei, Qinghong Gao, Yiqing Guo participated in collecting data and drafted the manuscript. Lanyan Wu and Xiangjian Wang carried out the histopathological examination and immunohistochemical staining. Hongmei Zhou and Chuanxia Liu helped to revise the manuscript. All authors read and approved the final manuscript.

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