Screening for Oral Cancer Using Brush Biopsies and DNA Image Cytometry – A Review

Pranjali Verma [1], Shikha Kumari Rajak [1]

Abstract

For the prompt cytologic diagnosis of oral cancer, non-invasive brush biopsies play a crucial role. Suspicious lesions in patients can be confirmed for malignancy using Deoxyribose Nucleic Acid Image Cytometry (DNAIC), offering a comprehensive diagnostic approach. The enhanced brush biopsy collects diagnostic material across different layers of the damaged epithelium, providing a non-invasive screening method. Despite the limitation of acquiring cells mainly from the superficial and intermediate layers of oral mucosa, DNAIC-DCNN utilizes Deep Convolutional Neural Network (DCNN) for widespread oral cancer detection. Semantic division is performed through the bottom branches, allowing for targeted medication to modify cancer cell features and inhibit proliferation. Targeted drugs, such as Cetuximab, can be used alone or in combination with chemotherapy and radiation treatment for specific patients with oral cancer. The non-invasive and accurate diagnostic capability of DNAIC in conjunction with cytology has demonstrated high precision and accuracy in identifying oral malignant squamous cell transition within epithelial tissues.

Keywords: Deoxyribose Nucleic Acid Image Cytometry, Deep Convolutional Neural Network, Brush Biopsies, Oral Cancer.

Significance | A non-invasive DNAIC-DCNN for accurate oral cancer diagnosis, offering targeted medications for effective treatment.

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1. Introduction

Early detection of oral cancer and precursors is crucial for effective treatment. Various techniques, including DNAIC and improved brush biopsy approaches, enhance diagnostic sensitivity. Oral brush biopsy is a painless, chair-side test, useful for locating potential dysplasia in red and white oral lesions. Dentists play a key role in oral cancer prevention through regular screenings, and additional tests like brush biopsy aid in identifying abnormal cells. The brush biopsy, utilizing a soft-bristled brush, is considered the gold standard for detecting oral cancer and precancerous lesions. Advanced techniques, such as DCNN, contribute to high recognition rates for mouth cancer images. Timely diagnosis through cytology tests and core biopsy is essential for determining malignancy and guiding treatment decisions. While the brush biopsy is a valuable tool, recognizing its limitations is crucial for effective implementation in dental practice.

The early detection of oral cancer and its precursors, such as precancerous lesions, is crucial for timely intervention and treatment (Idrees, M., 2022). A specialized technique called DNAIC, capable of identifying chromosomal aneuploidy equivalents through cytometry, has shown improved diagnostic sensitivity and specificity for early oral cavity lesion detection (Datta, M., 2022). Utilizing an enhanced brush in the biopsy process, this method ensures comprehensive transepithelial biopsy collection from basal, intermediate, and superficial layers of lesions (Yang, G., 2022).

The oral brush biopsy serves as a non-invasive screening method for individuals at high risk of developing mouth cancer, harvesting cells from deeper layers of the oral mucous barrier (Errazquin, R., 2023). This painless chair-side test is effective in identifying

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common tiny red and white oral lesions, aiding in the identification of potential dysplasia (Sayal, L., 2023). With multiple cutting surfaces, including a flat end and circular border, the brush biopsy is commonly employed for diagnosing precancer and cancer in various organ systems (Ribeiro, M. G. M., 2023).

Early detection of oral cancer is enhanced by employing deep learning techniques such as DCNN, which achieves a high recognition rate using ideal theoretical characteristics of mouth cancer images (Hemalatha, S., 2022). Regular oral cancer screenings by dentists contribute to prevention and improved treatment outcomes, often involving additional tests to identify abnormal cells in the mouth (Mosaddad, S. A., 2023).

Biopsy procedures, including tissue extraction and malignancy testing, play a crucial role in diagnosis. Core biopsy, more reliable than needle biopsy for soft tissue mass diagnosis, aids in determining tumor malignancy, establishing a definitive diagnosis, and guiding treatment decisions (Hu, Y., 2022). Brush biopsy tests excel in accurately identifying oral lesions as benign, and importantly, detecting precancerous and cancerous tumors even when not clinically suspected (Abati, S., 2020). Using a soft-bristled brush for cytological analysis, this method is considered the gold standard for detecting oral cancer and precancerous lesions in the mouth (Parakh, M. K., 2020) (Velleuer, E., 2020). When effectively implemented and embraced by dental practitioners, oral exfoliative cytology can detect malignant and premalignant lesions, as well as certain viral and fungal infections, even at advanced stages (Li, C., 2020).

The brush biopsy emerges as a painless and minimally invasive procedure for collecting oral cancer specimens, utilizing a brush to quickly rule out atypical hyperplasia, categorizing malignancy results as adverse, abnormal, or positive. The combination of DNAIC-DCNN presents a non-invasive, objective, and reproducible diagnostic technique in exfoliative cytology, effectively recognizing both oral malignant and potentially malignant conditions. Given that more than half of individuals with oral cancer have a disease that spreads during diagnosis, the screening process becomes crucial for swiftly detecting potentially malignant cancers, ensuring effective and timely treatment.

2. Literature Review

Eunike Velleuer MD and colleagues (2020) conducted a thorough examination of the population, revealing that individuals with Fanconi anemia face a heightened risk, a significantly accelerated onset, and fewer therapeutic options for oral Squamous Cell Carcinoma (SCC) treatment (Velleuer, E., 2020). The current study validated the diagnostic accuracy of an alternative preventive method employing oral brush biopsy. Patients undergoing brush biopsy cytology, manifesting visible mouth cancers and precancerous growths, benefit from a painless diagnosis of SCC and precancerous lesions. Positive cytology and the absence of DNA aneuploidy conclusively indicate high-grade oral epithelial dysplasia or SCC, thereby minimizing the need for invasive diagnostic biopsies.

Chenxi Li et al. (2020) presented contemporary data on the diagnostic utility of aneuploidy with DNA image cytometry (DNAIC), utilizing collection size and variable aneuploidy classification criteria for Oral Potentially Malignant Diseases (OPMD) brushings (Li, C., 2020). The objective is to evaluate the diagnostic accuracy of DNAIC in identifying OPMD with dysplasia and malignancy by determining optimal DNA content cut-off values. Each patient's brush and biopsy samples were analyzed using DNAIC and histological analysis. When the area under the curves and positive predictive value are sufficiently high, a minimum of a solitary aneuploid cell with a DNA index may be used to diagnose OPMD-related dysplasia.

Madhurima Datta et al. (2021) elaborated that the likelihood of advancement increases with the degree of dysplasia; however, the oral health risk assessment of Mild to moderate dysplasia, referred to as Low-Grade Dysplasia (LGD), can be challenging, as previously discussed (Datta, M., 2021). Additionally, dysplasia grading is subjective and may vary based on the site of the lesion biopsied. Lesion brushing cells analyzed for oral LGDs with a high DNA ploidy and chromatin organization score exhibited a high propensity to progress. According to a multivariate analysis, high-risk DNA brushing predicts disease progression more accurately than dysplasia grade or lesion clinicopathologic features. DNAIC can detect high-risk lesions decades, and potentially decades, before observable changes, accomplishing this without causing significant discomfort to the patient.

A few drawbacks of using DNA image cytometry with brush biopsies for oral cancer screening pose challenges in the modern day (Remmerbach, T. W., 2003). Brushings for DNAIC offer an advantage over biopsy tissue as cells can be acquired non-invasively for screening. However, cell preparation from brushings may be insufficient and restricted to the superficial or intermediate layers of the oral mucosa, making the detection of modest dysplastic alterations challenging. Velleuer's approach (Velleuer, E., 2020) aims to overcome the drawbacks identified by Li, Datta, and others and is compared with the proposed method DNAIC-DCNN.

3. Advancements in Non-Invasive Oral Cancer Detection and Epigenetic Factors

Brush biopsies offer a non-invasive and significant method for the early cytologic detection of oral cancer. The utilization of DNAIC-DCNN (Deep Convolutional Neural Network) serves as a complementary tool to confirm cytologic diagnoses or suspicions of malignancy in patients with worrisome lesions. Metabolites like

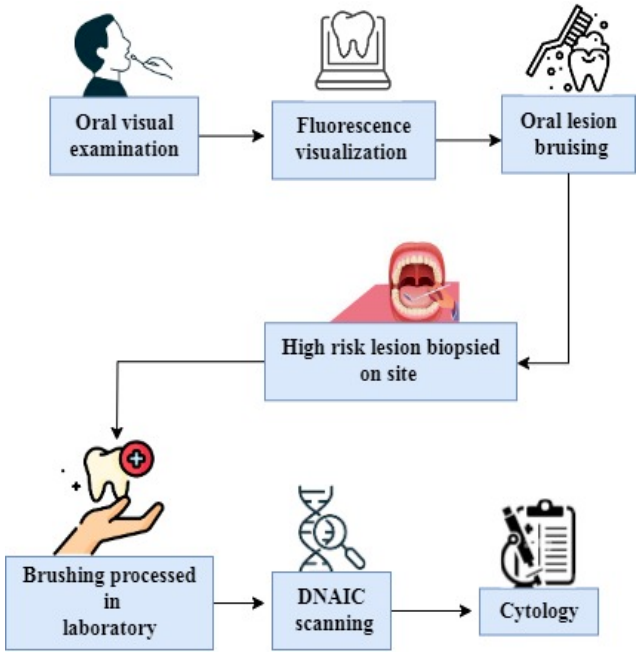

Figure 1. Oral cancer using brush biopsies and DNAIC

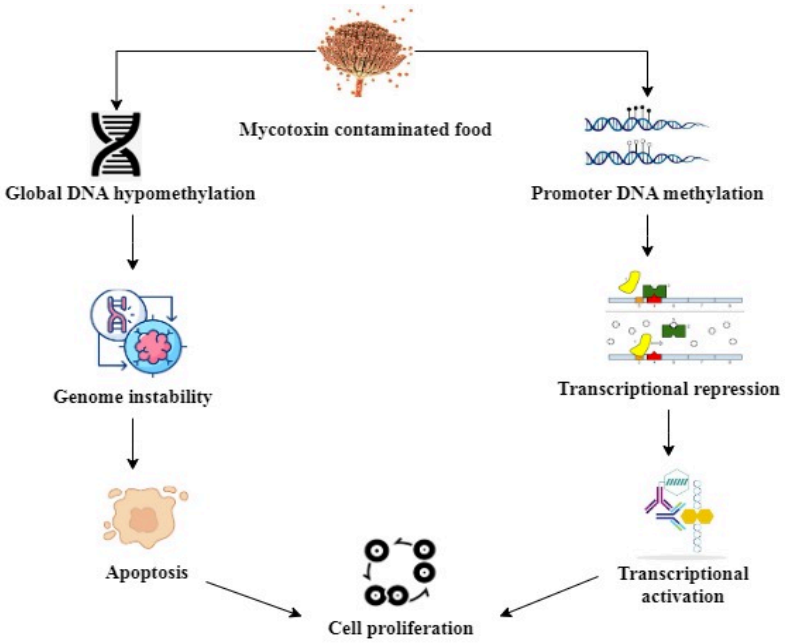

Figure 2. The effect of mycotoxins on DNA methylation and cancer

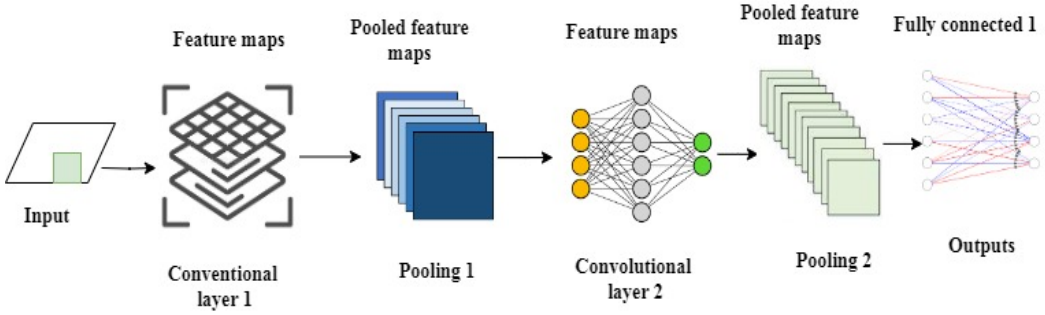

Figure 3. Architecture of DCNN

lactic acid and valine, along with amino acids and other compounds, have shown promise as indicators of oral cancer in early stages. Longitudinal investigations and screenings in large populations can enhance the effectiveness of DNAIC-DCNN using brush biopsies for oral cancer detection. The integration of auxiliary methods, such as fluorescence visualization, has proven successful in community oral cancer screenings. Results suggest that DNAIC and cytology, in addition to white light screening, provide a non-invasive strategy for identifying high-risk forms of oral cancer. The link between DNAIC damage and external factors like radiation and reactive oxygen species is explored. Smoking, a significant contributor to head and neck malignancies, is strongly linked to oral and throat cancer. Lifestyle modifications play a crucial role in cancer prevention, and evidence suggests that the epigenome can be influenced by micronutrients, bioactive chemicals, and mycotoxins in the diet.

Brush biopsies, which circumvent the need for skin incisions, play a pivotal role in the cytologic detection of early-stage oral cancer. For patients with concerning lesions, the application of DNAIC-DCNN can serve to confirm cytologic diagnoses or suspicions of malignancy. Research indicates that metabolites such as lactic acid and valine can act as indicators for oral cancer. Amino acids like glycine, proline, citrulline, and ornithine have also been linked to oral cavity squamous cell cancer in its initial stages. While DNA aneuploidy is acknowledged as a cancer risk factor, there is limited evidence supporting the efficacy of DNAIC-DCNN using brushings as an effective and complementary method for oral cancer screening. Longitudinal studies and screenings of extensive populations are essential for yielding more substantial results.

In Figure 1, auxiliary methods like fluorescence visualization and DNAIC were employed in community oral cancer screenings conducted in nearby areas. The results indicated that DNAIC and cytology functioned as a non-invasive strategy for identifying high-risk oral cancer forms, complementing white light screening. Screening biopsy cells for genetic alterations that indicate the type of cancer being treated is integral to this process (Kämmerer, P. W., 2013). The activation of oncogenes, suppression of tumor suppressor genes, and epigenetic changes, as depicted in Figure 2, are continuous and progressive events in cancer development. Modifying lifestyle can significantly contribute to preventing illness and extending the lives of cancer patients. The epigenome's susceptibility to micronutrients, bioactive chemicals, and mycotoxins in the human diet, with implications for cancer prevention or development, is supported by evidence (Datta, M., 2019). The potential hazards of varied quantities of these substances and their interaction with existing anti-cancer treatments are drawbacks, as illustrated in Figure 2 (Xiao, X., 2015).

Additionally, Figure 3, portrayed as a deep convolutional neural network (DCNN), is commonly employed for pattern recognition in video and still images. Derived from standard artificial neural networks, DCNNs utilize a three-dimensional neural design modeled after animals' visual brains. Although primarily applied to object recognition and image classification, these networks are increasingly used in natural language processing. The success of DCNNs relies on their parameter-heavy architectures and the ongoing advancements in powerful graphics processing units across diverse fields.

4. Non-Invasive Oral Cancer Detection through DNAIC Brush Biopsies

DNAIC brush biopsies present a non-invasive alternative for oral cancer screening, allowing the collection of cells from the oral mucosa without the need for tissue biopsy. However, challenges arise in detecting subtle dysplastic alterations, primarily due to the limited cell preparation depth in brushings, mainly accessing the outermost and middle layers of the mucosa. Despite this limitation, oral brush biopsies are extensively used for detecting oral cancer and precancerous tumors, providing a low-discomfort and low-blood-loss procedure. The dataset description emphasizes the urgent need for better early diagnosis and preventive treatment to reduce the over 50% death rate from oral cancer. An initial quantitative shotgun proteomics study was conducted on oral brush biopsies using mass spectrometry, aiming to analyze proteins extracted from normal tissue, oral premalignant lesions, oral squamous cell carcinoma, and matched control tissue. Results indicated decreased abundance of the leukocyte protease inhibitor protein across repeated proteomic datasets. DNAIC brush biopsies offer a promising avenue for non-invasive oral cancer detection, with the potential to enhance early diagnosis and preventive treatment strategies.

5. Conclusion:

Oral brush biopsy emerges as an efficient technique for early cancer diagnosis in private dentistry clinics, addressing the limited expertise of practicing dentists in identifying varied oral squamous cell carcinomas. Dentists, dental surgeons, and general practitioners can readily perform oral brush biopsies, making it a practical tool for early detection.

The clinical significance lies in the ability of oral brush biopsies, coupled with DNAIC, to offer diagnostic accuracy comparable to traditional biotic histology. Combining exfoliative cytology with DNAIC enhances sensitivity, making it a reliable method for diagnosing oral squamous cell carcinomas. Oral cancer screening, facilitated by DNAIC-DCNN, aims to identify potential precancerous lesions in their earliest, highly treatable stages.

Brush cytology is highlighted for its high accuracy, specificity, positive predictive value, and negative predictive value in diagnosing oral squamous dysplasia. The cost-effectiveness and simplicity of oral brush biopsies make them ideal for mass screening, providing an effective, non-invasive automated method for oral cancer screening. This approach aids in earlier detection, potentially reducing the need for invasive biopsies and improving outcomes in the treatment of oral cancer.

Author Contributions

P.V. and S.K.R. conceptualized, wrote and reviewed the paper.

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None declared.

Competing financial interests

The authors have no conflict of interest.

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Pranjali Verma pioneered the application of non-invasive brush biopsies and Deoxyribose Nucleic Acid Image Cytometry (DNAIC) for oral cancer diagnosis, enhancing diagnostic precision.

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Shikha Kumari Rajak focused on the integration of Deep Convolutional Neural Network (DCNN) in oral cancer detection, proposing targeted medications like Cetuximab for effective treatment strategies.

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