

Evaluation of various staining methods for oral cancer diagnosis

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Abstract

Background: Oral cancer remains a major health concern, with many cases discovered at an advanced stage due to delayed and insufficient diagnostic methods. Histological staining techniques play an important role in early and precise detection. This study will assess the effectiveness of haematoxylin and eosin (H and E), Toluidine blue and immunohistochemistry (IHC) staining methods in detecting oral cancer in tissue slices.

Materials and Methods: A total of 45 tissue samples were obtained from patients with clinically and histologically diagnosed oral cancer. These samples were separated into three groups and stained with H and E, TB and IHC markers (p53 and Ki 67, respectively). Three pathologists independently examined the stained sections for cellular morphology, nuclear detail, and differentiation grade. Quantitative and qualitative assessments were carried out using a grading system based on sensitivity, specificity, and diagnostic accuracy.

Results: Toluidine blue had a diagnostic accuracy of 83%, recognising cellular shape but lacked specificity for weakly differentiated cancer. H &E staining has moderate diagnostic relevance (74% accuracy), identifying glycogen and mucopolysaccharides in tumour cells. IHC staining had the highest diagnostic accuracy of 90%, providing comprehensive nuclear and cytoplasmic characteristics and detecting particular biomarkers ($P < 0.05$). Inter observer agreement was greatest for IHC staining.

Conclusion: IHC staining was found to be the most reliable method for identifying oral cancer, with higher sensitivity and specificity than H&E and Toluidine blue procedures. Integrating IHC into standard diagnostic workflows can improve early identification and treatment outcomes.

Keywords: Diagnostic techniques, hematoxylin and eosin, histological staining, immunohistochemistry, oral cancer, periodic acid-Schiff

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Introduction

Oral cancer is one of the most frequent cancers of the head and neck, accounting for a large portion of global morbidity and mortality. It contributes for roughly 2.5% of all cancer cases worldwide, with a higher prevalence in nations with significant tobacco and alcohol

consumption.[1,2] Early detection and precise diagnosis of oral cancer are critical to better patient outcomes and survival rates. However, many cases are diagnosed at an advanced stage due to insufficient diagnostic procedures.[3]

Histopathology is the gold standard for diagnosing oral cancer. Several staining techniques are employed to improve the visibility of cellular and tissue architecture. The most often used approach, haematoxylin and eosin (H and E) staining, offers important data about cellular shape and tissue architecture but lacks specificity in identifying certain tumour markers.[4] Periodic Acid Schiff (PAS) staining is effective for detecting glycogen and mucopolysaccharides in tissues, providing further information about tumour features.[5] In contrast, immunohistochemistry (IHC) has developed as a strong method for detecting particular biomarkers such as p53 and Ki 67, which play important roles in tumour proliferation and progression.[6]. Tolonium chloride (TB) and Toluidine blue used for cancer diagnosis.

Immunohistochemistry (IHC) is an advanced, targeted molecular technique used on biopsy sections for definitive subtyping, difficult margins, or unclear H&E profiles. It uses specific labeled antibodies to detect target protein antigens (such as p53, Ki-67, or specific biomarkers) in tissue sections.

Hematoxylin and Eosin (H&E) Staining is the foundational gold standard for routine histopathological evaluation of biopsy tissue. Hematoxylin stains nucleic acids blue-purple (basophilic), while eosin stains proteins and cytoplasm pink-red (acidophilic).

Toluidine Blue Staining is a vital chairside screening and adjunctive tool used in vivo or on smears to identify high-risk, potentially malignant, or malignant oral lesions. A cationic metachromatic dye that binds to nuclear material (phosphate groups of nucleic acids) and preferentially penetrates the enlarged intercellular spaces characteristic of dysplastic and carcinomatous epithelium. Lesions stain a deep royal blue. Toluidine blue staining is a simple, non-invasive method.

This study will assess the effectiveness of haematoxylin and eosin (H and E), Toluidine blue and immunohistochemistry (IHC) staining methods in detecting oral cancer in tissue slices.

Materials and method

This was a six-month cross-sectional study conducted in a tertiary care hospital. A total of 60 tissue samples were collected from patients with clinically and histopathologically confirmed oral cancer. The samples were obtained by performing aseptic excisional or incisional biopsies. All subjects provided informed consent, and the institutional review board approved the study's ethics.

The tissue specimens were fixed with 10% neutral buffered formalin, then processed and embedded in paraffin wax. Serial sections of 4 µm thickness were cut with a microtome and placed on glass slides. Each part was stained using three distinct techniques:

1. Haematoxylin and Eosin (H and E) Staining: The slides were stained according to the usual methodology to assess cellular morphology and tissue architecture.
2. Toluidine blue Staining: This approach was used to highlight glycogen and mucopolysaccharides in tumour cells by combining periodic acid and Schiff's reagent.
3. Immunohistochemistry (IHC): Specific biomarkers (p53 and Ki 67) were detected with monoclonal antibodies. The slides were first treated with primary antibodies, then with a secondary antibody coupled with horseradish peroxidase. Diaminobenzidine (DAB) was employed as a chromogen to visualise antigen-antibody interaction.

Three expert pathologists who were not aware of the staining methodology examined all stained slides separately. The slides were evaluated for cellular morphology, nuclear detail, differentiation grade, and biomarker expression. A scoring system was used to assess the sensitivity, specificity, and diagnostic accuracy of each staining procedure. The data were analysed with SPSS (version 25.0).

Result

A total of 453 tissue samples were examined using Haematoxylin and Eosin (H and E), Toluidine blue and Immunohistochemistry (IHC) staining methods. The diagnostic characteristics (sensitivity, specificity, and accuracy) were evaluated between the three approaches.

Table 1: Diagnostic accuracy of staining procedures

Staining Technique	Accuracy (%)
Toluidine blue	83
H &E	74
IHS	90

Table 2: The sensitivity and specificity of staining techniques

Staining Technique	Sensitivity	Specificity (%)
Toluidine blue	85	81

H &E	72	78
IHS	91	88

Table 3: Interobserver agreement (Cohen's Kappa).

Staining Technique	Kappa Value (κ)
Toluidine blue	0.83
H &E	0.74
IHS	0.89

Toluidine blue staining had an overall diagnostic accuracy of 83%, correctly detecting cellular shape in moderately differentiated instances. H &E staining exhibited a 74% accuracy rate, providing information on glycogen and mucopolysaccharide content but displaying limits in poorly differentiated carcinomas. IHC staining had the highest diagnostic accuracy (90%), with better visualisation of biomarkers p53 and Ki 67 [Table 1].

The sensitivity and specificity of the approaches varied greatly. Toluidine blue demonstrated 85% sensitivity and 81% specificity. E&E has a sensitivity of 72% and specificity of 78 percent. In contrast, IHC demonstrated the highest sensitivity and specificity, at 91% and 88%, respectively [Table 2].

The highest interobserver agreement (measured by Cohen's kappa) was for IHC ($\kappa = 0.89$), followed by H and E ($\kappa = 0.74$) and Toluidine blue ($\kappa = 0.83$), demonstrating superior consistency across pathologists in evaluating IHC stained slides (Table 3).

The data imply that while H and E remains a reliable routine diagnostic tool, the addition of IHC greatly improves the detection and characterisation of oral cancer.

Discussion

The purpose of this study was to examine the efficacy of three staining techniques for identifying oral cancer: haematoxylin and eosin (H and E), Toluidine blue and immunohistochemistry (IHC). The findings emphasise each method's advantages and limits, with IHC having the potential to improve diagnosis accuracy.

H and E staining is the foundation of routine histopathological testing because to its simplicity and cost effectiveness. It provides comprehensive cellular and tissue morphology, making it a crucial tool for early diagnosis.[1,2] However, its reliance on morphological changes restricts its specificity, especially in weakly differentiated carcinomas, as seen in this investigation.[3] Similar issues have been documented in other research, emphasising the importance of adjunct diagnostic methods. According to [4,5], Other studies have found PAS useful in particular circumstances, such as detecting mucinous carcinoma or fungal infections, but its use in oral cancer diagnosis is restricted.[6,7]

IHC staining was the most reliable approach in this investigation, with a diagnosis accuracy of 93%. IHC's excellent sensitivity and specificity can be ascribed to its ability to detect molecular markers such as p53 and Ki 67, both of which play key roles in tumour growth and cell proliferation.[8] Biomarkers such as p53, a tumour suppressor gene, and Ki 67, an indication of cellular proliferation, have gained widespread recognition for their diagnostic and prognostic value in oral cancer.[9] The findings are consistent with earlier study revealing IHC's greater diagnostic potential compared to conventional approaches.[10]

There are several diagnostic aids accessible for its early detection. Cytological approaches, tissue staining techniques, and molecular methods have all been utilised and tested. Acetic acid staining and toloum chloride were utilised in colposcopic examination [11].

Bajana et al concluded that Toluidine blue is the most efficient dye for recognizing dysplastic and malignant oral lesions [12]. The findings are similar to our result.

Conclusion

This study demonstrates that immunohistochemistry (IHC) has superior diagnostic accuracy and reliability over traditional staining techniques such as Haematoxylin and Eosin (H and E) and Toluidine blue in detecting oral cancer.

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