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Comparative analysis of oral epithelial malignancies using routine stain and modified Cajal's trichrome stain: An histopathological study

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ABSTRACT

Introduction: Diagnosis of initial epithelial pathology maybe difficult in Oral Squamous Cell Carcinoma (OSCC), early invasive squamous cell carcinoma and other atypical epithelial malignancies, under routine Haematoxylin and Eosin (H and E) stain. In this context, an appropriate differential stain capable of distinguishing epithelial cells in the connective tissue may well prove to be a valuable aid. In the present study, we endeavor to use Modified Cajal's trichrome stain for making diagnosis easier without resorting to expensive diagnostic aids. Aim of present study is to assess both the epithelial and connective tissue elements in early invasive carcinoma, verrucous carcinoma and oral squamous cell carcinoma in tissue sections stained with modified Cajal's trichrome stain (MCTS) and to compare it with H and E-stained sections.

Materials and Methods: Formalin-fixed, paraffin-embedded tissue blocks of early invasive SCC (n = 10) Verrucous carcinoma (n = 10) OSCC (n = 10) were stained with MCTS and H&E. The sections were compared based on set histopathological criteria.

Results: In SCC cases stained with MCTS, invasion into connective tissue and keratin pearls were strikingly evident. Depth of invasion could be more accurately determined. Thus, CTS is a good differential stain, clearly delineating the epithelial elements from the connective tissue elements visually.

1. Introduction

Squamous cell carcinoma (OSCC) is the most common type of oral cancer, accounting for over 90% of all cases reported. Oral cancer is a significant human disease. OSCC is one of the most prevalent epithelial cancers, has a significant morbidity and mortality, and its etiology is multifactorial. Although OSCC can develop on its own, the majority of cases are preceded by clinically evident changes in the oral mucosa that have the potential to transform into cancer. Together, these lesions are categorized as disorders that could be cancerous.[1]

These lesions require histopathological examination for confirmation of diagnosis and progression to malignancy because their clinical appearance can range from benign to sinister. The histopathological examination of the affected tissue determines the final diagnosis of a clinically suspicious malignancy.[2]

The early-stage, relatively "thin" tumor known as microinvasive squamous cell carcinoma, which does not invade deep tissues, is one aspect of OSCC that needs to be taken into consideration. Microinvasive oral squamous cell carcinoma has no clear definition, but there are classification systems for similar lesions in other places, such as the presence of microinvasion in cervical cancer. An early-stage, relatively thin tumor confined to the papillary lamina

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propria by the depth of the rete process is known as microinvasive OSCC. Due to greater variations in epithelial thickness, the depth, which typically ranges from 0.5 to 2 mm, must be measured from the non-neoplastic surface epithelium that is adjacent. A microinvasion is also one with an irregular infiltrative border, usually accompanied by a reactive desmoplasia rather than a pushing type of hyperplastic epithelium expansion.[3]

It is necessary to rule out sectioned rete process, which can be confirmed by the presence of cellular atypia in the invaded epithelial component. However, difficulties may arise when the invasion is very minimal and is masked by the presence of dense inflammation. Diagnosis of microinvasive OSCC requires careful evaluation of the entire tissue section. Islands of epithelium within the lamina propria should be regarded with suspicion. After that, the pathologist must locate the breach in the basement membrane, which may not always be possible in routine H and E stained sections. Special stains and histochemical methods can be used to confirm the diagnosis in these situations as useful adjuvants.[4] Special stains can be a simple, cost-effective way to find pathologies before using advanced molecular markers. Histopathologists have long been drawn to stains that can simultaneously distinguish various tissue components in a histological section, and there are numerous histological methods that give varying colors.

Cajal's trichrome stain is one such ancient special stain that we have utilized in the current study. Ramon Y. Cajal introduced it in 1897, and Gallego modified it in 1919 to become Modified Cajal's Trichrome stain (MCTS). With a variety of color tones, this differential stain visually distinguishes between connective tissue and epithelial components.

2. Aim

To assess viability of Altered Cajal's Trichrome Stain (MCTS) corresponding to Haematoxylin and Eosin in identification of microinvasion.

3. Materials and Methods

The study will be conducted at the institute's department of Oral Pathology & Microbiology to evaluate the epithelial and connective tissue components of early invasive, verrucous, and oral carcinoma tissue sections.

Histopathologically diagnosed cases of Early invasive SCC, Verrucous carcinoma, and oral squamous cell carcinoma will be included in the study population.

Formalin-fixed, paraffin-embedded tissue blocks of 10 early invasive SCC, 10 OSCC, and 10 verrucous carcinoma were stained with MCTS and H&E. The sections were compared based on established histopathological criteria.

3.1. Inclusion criteria

1. Histopathologically diagnosed cases of OSCC

3.2. Methods for Cajal trichrome staining modified by Gallego

1. Formalin-fixed, paraffin-embedded tissue blocks must be cut into four-micron-thick sections. After that, the sections must be deparaffinized and hydrated, stained with Ziehl's acetic fuchsin for two minutes (Ziehl's fuchsin - 10 drops, acetic acid - 1 drop, distilled water 10 cc), washed in water, differentiated in formalin-acetic acid solution for five minutes (formalin -2 drops, glacial acetic acid - 2 drops, distilled water 10 c).

The following criteria were used to compare the modified Cajal's trichrome-stained sections with H and E:

1. Cytoplasmic and nuclear details;
2. Cell stratification;
3. Dysplasia screening;
4. Dysplasia grading;
5. Depth of invasion;
6. Differentiation of epithelial and connective tissue components;
7. Ease of use; and
8. Economics.

4. Results

Cajal's trichrome stain is a good differential stain for identifying connective tissue stroma epithelial cells. Nuclei stained red, epithelial cytoplasm stained pink-green, collagen fibers stained blue, muscle stained olive green, and keratin and red blood cells stained grass green with Cajal's trichrome stain.

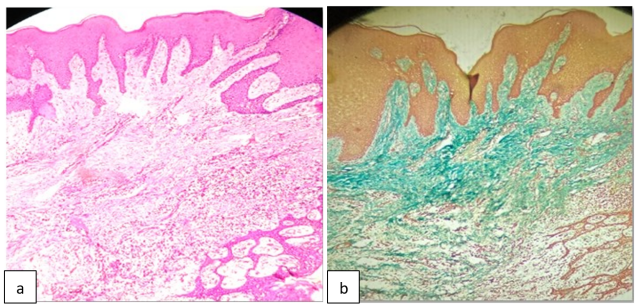

Fig. 1: a): Epithelial cytoplasm- Pink, Nuclei- Dark blue, Collagen fibres – Pink; **b)**: Epithelial cytoplasm- Pink-green, Nuclei- Red, Collagen fibres- Blue green

5. Discussion

Both proper treatment planning and determining a patient's prognosis depend on an accurate diagnosis and

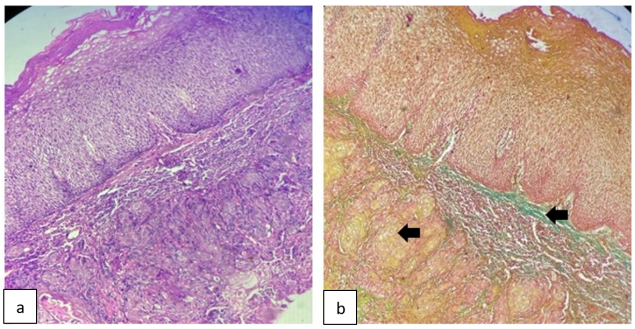

Fig. 2: Oral squamous cellcarcinoma; **a, b**): H&E stain, Invasion of tumor cells into connective tissueand the presence of keratin pearls were strikingly evident in (**b**)

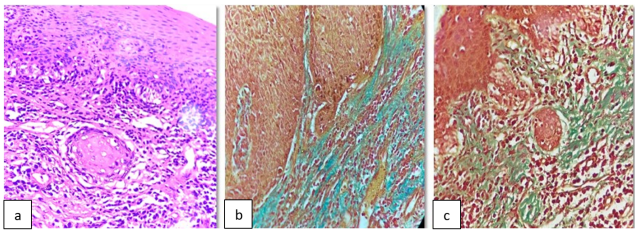

Fig. 3: Early invasive SCC; **a**):, H&E stain; **b**): MCT stain, provides remarkable differentiation between epithelial and connective tissue element; **c)**: MCT stain, infiltration of malignant cells in connective tissue stroma is easily identifiable

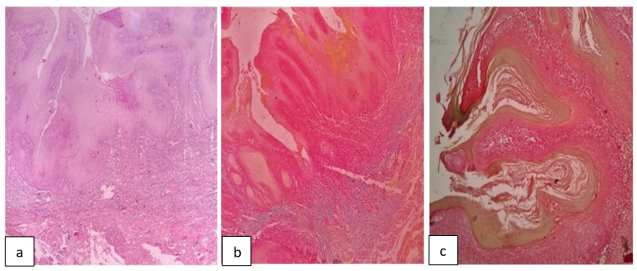

Fig. 4: Verrucous carcinoma, **a**): H&E stain; **b, c**): MCT stain, Epithelial and connective tissue is easily differentiated and keratin is strikingly visible

Table 1: Comparison of Modified Cajal's trichrome stain and H & E stain

Histopathological features	Modified Cajal's trichrome Stain	H&E Stain
Differentiation Between epithelium & Connective tissue	+++	++
Cytoplasmic details	++	++
Nuclear details	++	+++
Cell Stratification	++	+++
Grading of dysplasia	++	+++
Assessment of invasion	+++	+
Ease of use	+++	++
Economics	+++	++

+ Average, ++ Good, +++ Excellent, H & E –Hematoxylin & Eosin

prompt detection of epithelial pathologies. Histological examination of formalin-fixed paraffin embedded tissues stained with hematoxylin and eosin (H and E) remains the foundation of contemporary cancer diagnosis and pathological staging in routine practice, despite the growing use of molecular biology in the study of oral squamous cell carcinoma (OSCC).

When a case of "early SCC" arises, the most difficult decision a pathologist must make is whether or not frank invasion is present. In this regard, thorough consideration is required for the instances of micro-invasive SCC. In the early stages of tumor progression, micro-invasive SCC is regarded as a relatively "thin" tumor due to its lack of deeper invasion into connective tissue.

Histopathological diagnosis of initial epithelial pathology may be challenging, despite the fact that hematoxylin and eosin staining remains the foundation of contemporary cancer diagnosis. An appropriate differential stain that is capable of distinguishing epithelial cells in the connective tissue may very well prove to be a useful aid in this context because of the inflammatory reaction's masking of small and obscure invasive components as well as the basement membrane. The goal of this study is to make use of Modified Cajal's trichrome stain to quickly and easily diagnose microinvasive squamous cell carcinoma.

The histopathology laboratory rarely employs the Cajal trichrome stain, which was first described by Cajal and later modified by Calleja and Gallego. Castroveijo wrote a review of Cajal trichrome stains in 1932, and in it, he went into great detail about the various staining methods and principles. His research shows that this stain is one of the best for studying corneal pathology and diagnosing tumors like fibrosarcoma, neurofibroma, and myofibroma. He explained that this stain has varying hues depending on the degree of keratinization and is especially useful in tumors where the connective tissue framework and epithelial cells are associated.[5] This provided the foundation for the hypothesis that this stain will be especially helpful in the diagnosis of early micro-invasive SCC.

Ziehl's acetic fuchsin leaves a dark pink stain on all of the structural components. Only the acidic tissues (such as nuclei) retain the fuchsin color after differentiation with formol acetic acid removes the stain color from most tissue components. The basic tissues absorb the blue color from the counterstain picroindigocarmine, while the more acidic tissues gradually absorb the green color. Using formol acetic acid, the process of "viro-fixation" preserves only the nucleus's fuchsin color. Gallego added formaldehyde as a "viro-fixing fluid" and acetic acid as the differentiating fluid to the original Cajal method. When acetic acid is added to fuchsin and formalin, differentiation is enhanced. Using this property of CTS, epithelial cells can be differentiated into connective tissue, facilitating subsequent diagnosis of early invasive carcinoma.

The current study revealed that MCTS gives distinct colors to various tissue components. The control was gingival tissue, which was stained with both H&E and MCTS. Figure 1 a and b show how MCTS color various connective tissue and epithelial elements. As a result, it aids in the diagnosis of early invasive carcinoma and the detection of tumor buds in connective tissue (Figure 2 a and b). In cases of verrucous carcinoma, it is also helpful in identifying keratin plugging (Figure 4 a, b). This was in line with the findings of a 2017 study that compared the efficacy of modified Cajal's trichrome stain (CTS) to haematoxylin and eosin for the examination of epithelial dysplasia, carcinoma in situ, microinvasive SCC, frank SCC, and SCC in lymph nodes. And discovered that invasion into connective tissue and keratin pearls were strikingly evident in SCC cases stained with CTS. The invasion's depth could be determined with greater precision. The lymph node tumor cells had sharp contrast and were easy to see. According to some studies, this stain has varying hues depending on the degree of keratinization and is especially useful in tumors where epithelial cells and connective tissue framework are linked. Using MCTS, tumor cells in the lymph node could be easily identified, as stated by Sanjai et al.[6]

In a similar vein, Latha et al.[5] conducted research on the diagnostic accuracy of modified Cajal's Trichrome stain in cases of suspected microinvasion. They came to the conclusion that cases of severe dysplasia were straightforwardly diagnosed using modified CTS. Additionally, they found that both stains were moderately consistent in detecting microinvasion, with a statistically significant p value of 0.05 within a confidence interval of 95%.

A potential, cost-effective, and simple-to-use stain known as MCTS can be utilized in conjunction with microinvasion detection. In special circumstances where the invasive nature of the tumor is not readily discernible, it can also be used in situations requiring the screening of large samples of potentially malignant or malignant lesions.

6. Conclusion

Potential flaws in cancer diagnosis can be fixed by utilizing Cajal's trichrome as an additional histopathology stain. Cajal's trichrome may prove to be a useful addition to a pathologist's arsenal, despite the fact that H and E remains the gold standard for pathological analysis. To find a solution to the microinvasion conundrum using this differential stain, additional research with a larger sample size is required.

7. Ethical Consideration

Ethical clearance from Institutional Ethics Committee was obtained.

8. Source of Funding

None.

9. Conflict of Interest

None.

References

1. Alex S, Bhandare PR, Patankar SR, Sridharan G. Microinvasive oral squamous cell carcinoma- A clinicopathological study. *Med Res Arch*. 2017;5(7):1–11.
2. Pentenero M, Navone R, Motta F, Marino R, Gassino L, Broccoletti R, et al. Clinical features of microinvasive stage I oral carcinoma. *Oral Dis*. 2011;17(3):298–303.
3. Shankar A, Sridharan G. The dilemma of microinvasion. *Head Neck Oncol*. 2014;6(3):30.
4. Castroviejo R. Modifications of Differential Stains with Special Reference to the Trichromic Stain of Cajal. *Am J Clin Pathol*. 1932;2(2):135–40.
5. Cherian LM, Sasikumar D, Sathyan P, Sabu K. A Study on Diagnostic Accuracy of Modified Cajal's Trichrome Stain in Suspected Cases of Microinvasion. *Int J Sci Res*. 2019;10(1):4.
6. Sanjai K, Baker A, Reddy LP, Pandey B. Modified Cajal's trichrome stain as a diagnostic aid in the study of epithelial pathology. *Indian J Pathol Microbiol*. 2017;60(4):528–32.

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