Tumor Microenvironment & Its role In prognosis Of Oral Squamous Cell Carcinoma- A Clinico-pathologic study

Abstract:

Background: The challenges in evaluating oral squamous cell carcinoma primarily arise from its diverse histologic patterns. This variability is considered one of the reasons influencing prognosis. Special stains serve as valuable adjuncts to hematoxylin and eosin (H&E) staining.

Objectives: To investigate the role of mucin expression by mucicarmine stain in different grades of oral carcinoma.

Methodology: A total of 150 cases were subjected to mucicarmine staining. The staining patterns were observed in the stroma, extracellular matrix, and in areas surrounding tumor islands.

Result: Bright staining was more prominent in poorly differentiated oral squamous cell carcinoma than in moderately and well-differentiated cases. Statistically significant differences were observed among three grades of OSCC.

Conclusion: The pattern of mucicarmine staining varies among different grades of oral carcinoma, and less mucin expression relates to better prognosis.

Key-words: Extracellular matrix, H&E Stain, Metastasis, Mucicarmine stain, Special stain, Tumor Microenvironment.

Introduction:

The extracellular matrix (ECM) significantly influences tumor behavior and cellular function. Matrix metalloproteinases (MMPs), produced by cancer-associated fibroblasts and inflammatory cells, degrade the structural components of the ECM. This degradation facilitates increased tumor invasion, thereby affecting prognosis. The ECM is composed of collagen, elastin, and a viscoelastic ground substance made up of proteoglycans and glycoproteins. Collagen, the most abundant protein in the ECM, provides mechanical strength and maintains structural integrity. It acts as a primary barrier, resisting tumor cell infiltration. Approximately 300 proteins are present in the ECM in different proportions, including collagens, ground substance, elastin and glycoproteins in different proportions. The role of tumor micro-environment is that it contains many different cell populations, such as cancer-associated fibroblasts and various immune cells.

Despite modern interventions, the five-year survival rate for oral squamous cell carcinoma has improved only marginally over the past few decades, with recurrent disease observed in nearly 50% of patients. Treatment failure in head and neck cancers can be attributed to multiple factors that are difficult to predict, and the prognosis largely remains uncertain. Early detection of these lesions remains a diagnostic challenge for most clinicians, and ideal adjunctive tools are currently unavailable. Challenges in evaluating and diagnosing

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potentially malignant disorders of the oral cavity primarily arise from a lack of standardized criteria for histological grading. The term “special stain”traditionally refers staining other than H&E. Special stains are valuable tools for detecting and evaluating the role of the connective tissue stroma in various lesions of the oral cavity. It encompasses a wide variety of techniques used to visualize specific tissue structures, elements, or microorganisms not identifiable by H&E staining. Mucicarmine stain differentiates acid mucin from neutral mucin. It is also used to study salivary gland pathology and to detect benign changes in serous and mucous cells. Mucicarmine staining is employed to study changes in salivary tissues and is indicative of benign alterations in serous, mucous, and myoepithelial cells. However, its role in detecting malignant changes in oral tissues is not clear.

Materials and Methods:

The study group consisted consisted of 150 formalin-fixed, paraffin-embedded tissue sections, including 50 each of well-differentiated, moderately differentiated, and poorly differentiated squamous cell carcinoma of the oral cavity. The sections were preserved in formalin (Table 1). A detailed history, including relevant habits and clinical data, was obtained and recorded using a standardized proforma. Clinical diagnoses were confirmed through histopathological examination. Hematoxylin and eosin (H&E) staining was used for this

Condition	Cases	Average age	Age range	Site							Habits	
				Lip	Cheek	Gingiva	Tongue	Palate	FOM	Ridge	Y	N
Normal mucosa	50	30	20-40	4	26	4	6	4	2	4	30	20
WDOSCC	50	45	30-70	8	26	5	4	3	4	4	46	4
MDOSCC	50	52	32-64	10	23	5	3	2	3	4	41	9
PDOSCC	50	56	36-72	8	20	6	6	3	4	3	45	5

Table 1

Histological Staining:

Four-micron-thick tissue sections were obtained from formalin-fixed, paraffin-embedded tissue blocks. The sections were stained and examined using a conventional light microscope. Staining was evaluated in 10 randomly selected high-power fields, corresponding to 2 mm^2 of tissue. For hematoxylin and eosin (H&E) staining, the tissue sections were passed through a series of decreasing alcohol concentrations (100%, 90%, 80%, and 70%). The sections were stained with hematoxylin for 3–5 minutes. After washing, they were treated with 1% acid alcohol. Subsequently, the sections were stained with 1% eosin Y for 10 minutes, then washed and cleared in xylene. Mucicarmine staining was performed according to the standard protocol.

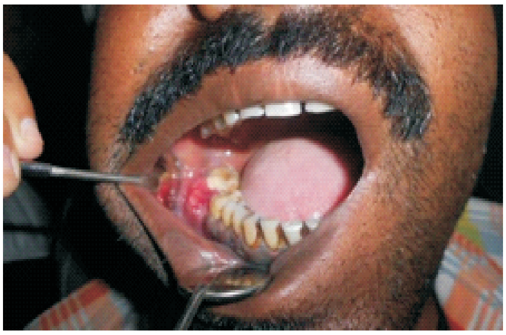

Fig 1: OSCC in posterior mandibular region

Evaluation of Staining:

The localization of the Mucicarmine stain was examined under high magnification. Tumor tissue from biopsy specimens was evaluated before selecting a final, representative section. The distribution of the stain within the tumor microenvironment was assessed, with findings recorded as (+) indicating the presence of stain and (-) indicating its absence. Intra-examiner and inter-examiner calibrations involving two examiners were conducted and verified to minimize discrepancies. To avoid inaccurate scoring, areas of intense inflammation and necrosis were excluded. The scoring was categorized as negative, light, or bright. Additionally, the scoring was divided in two sections: less than 50% of surface area positivity and more than 50% surface area positivity.

Results:

In well-differentiated oral squamous cell carcinoma, 12% of sections showed negative staining for mucicarmine, 52% showed light staining, and 36% exhibited bright staining. In moderately differentiated oral squamous cell carcinoma, 16% of sections showed negative staining, 28% showed light staining, and 56% showed bright staining. In poorly differentiated oral squamous cell carcinoma, 8% of sections showed negative staining, 20% showed light staining, and 72% exhibited bright staining.

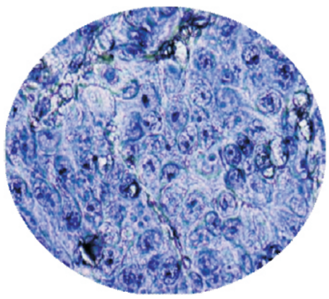

Fig. 2- Mucicarmine positivety seen in porrly differentiated OSCC

Statistical Analysis:

The relationship between two categorical variables was assessed using the Chi-square test. Inter-observer variations were also examined. Observations were tabulated and analyzed using statistical methods, including the Chi-square test, Fisher's Exact Test, and Cramer's V. Evaluation of staining intensity across different grades of OSCC revealed a highly significant p-value ($p < 0.001$).

Discussion:

The components of the surrounding stroma in oral tissues are important regulators of cancer development. These different regulators are: ability to escape apoptosis, neo-angiogenesis, deregulation of metabolism, and resist immune detection. These different phases lead to increased invasion and metastasis. Hematoxylin and eosin (H&E) staining is considered the gold standard in histology. Haematoxylin is a basic dye that stains basophilic, structures basophilic structures, such as and ribosomes, endoplasmic reticulum blue. reticulum, an acidic dye Eosin stains eosinophilic structures red. Periodic acid–Schiff (PAS) staining detects glycogen in tumors of the bladder, kidney, ovary, pancreas, and lung. It also visualizes basement membranes and is used to demonstrate hyphae and yeast forms of fungi in tissue samples due to the high carbohydrate content of their cell walls. Masson's trichrome is a three-color staining procedure widely used in histology to study muscular pathologies (such as muscular dystrophy), cardiac diseases, liver cirrhosis, and glomerular fibrosis. The mucicarmine technique involves carmine interacting with negatively charged connective tissue acid mucins, resulting in the staining of acidic mucins, while neutral mucins remain unstained. The reagents used include aluminum hydroxide, 50% alcohol, and aluminum chloride. This technique is employed to identify gastrointestinal disorders and certain fungal diseases, such as Cryptococcus. In present study, mucicarmine stain was used to examine changes in various tissue components in oral squamous cell carcinoma (OSCC). Staining intensity was greater in poorly differentiated OSCC compared to moderately and well-differentiated cases. Bright staining was observed in 72% of tumor islands in poorly differentiated OSCC. In well-differentiated OSCC, 36% of tumor islands showed strong staining intensity, while 46% exhibited strong staining in moderately differentiated OSCC. Also poorly differentiated OSCC, collagen bundles and the extracellular matrix (ECM) stained with strong intensity. These findings are consistent with a study by Patankar et al. (2016), which reported changes in the ECM among different grades of OSCC. According to their research, these changes result from enzymes released by tumor cells that modify the ECM to promote tumor survival. Additionally, collagen content was higher around tumor islands in well-differentiated OSCC compared to other grades. The reduction in collagen content in higher-grade tumors is attributed to alterations in the tumor microenvironment.

Conclusion:

Immunohistochemistry (IHC) is used to study proteins or changes in DNA/RNA sequences. Special stains are less expensive, readily available and are relatively easy to use. The prognosis was favorable in well differentiated OSCC with less positivity by mucicarmine. Our study reveals a definite relationship between different grades of oral squamous cell carcinoma (OSCC) and mucicarmine positivity.

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