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TO COMPARE THE EFFICACY OF HAEMATOXYLIN AND EOSIN (H AND E) AND PERIODIC ACID SCHIFF (PAS) TO IDENTIFY THE BREAK IN CONTINUITY OF BASEMENT MEMBRANE (BM) IN SQUAMOUS CELL CARCINOMA (SCC)

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Abstract:
Aim: To analyse the efficacy of H and E and PAS to identify the break in continuity of basement membrane in SCC.
Objective: To evaluate the efficacy of H and E and PAS to identify the break in continuity of basement membrane in SCC.
Materials and Method: This entire study was conducted in Saveetha Dental College and Hospitals, Department of Oral Pathology.Five OSCC tissue samples were collected from the archives of Saveetha Dental College and Hospitals, sectioned by a microtome and two set of slides were prepared for each tissue sample. Then the first set of slides were stained with H and E and the other set of smears were stained with PAS following the staining protocol.
Back Ground: Basement membrane (BM) is an electromicroscopically, amorphous, thick sheet of extracellular matrix molecules, upon which epithelial cells attach. Squamous cell carcinoma (SCC) is an invasive epithelial neoplasm with varying degrees of squamous differentiation and a propensity to early and extensive lymph node metastases, occurring predominantly in alcohol and tobacco-using adults in the 5th and 6th decades of life. In SCC, there is a break in the basement membrane continuity.
Reason: Although the pathological changes in the basement membrane can be detected very specifically by immunohis to chemical methods on paraffin-embedded tissue sections, the antikeratin-antisera are not economical and are time consuming. Hence if the efficacy of the standard stains like H and E and special stains like PAS can be assessed and compared, it could be very advantageous because of its low cost and simple handling technique.

Key Words: Basement Membrane, H and E, PAS & Squamous Cell Carcinoma (SCC).

Introduction:

Our oral cavity is lined by stratified squamous epithelium. Beneath this layer lies the basement membrane. The basement membrane seperatesthe epithelium and connective tissue layer of the oral mucosa[1]. It can be defined as a thick sheet of extracellular matrix molecules, to which the epithelial cells are attached[2,3]. When observed under the electron microscope, the basement membrane consists of two layers- the basal lamina and the underlying layer of reticular connective tissue[4,5]. This underlying connective tissue is attached to the basal lamina with collagen VII anchoring fibrils and fibrillin microfibrils. These two layers together are termed as the basement membrane [4,5]. The main function of the basement membrane is to provide structural support to the overlying epithelial cells along with other cell types like the mesothelial and endothelial cells. Apart from these, it also surrounds the adipocytes, muscles and Schwann cells[2,6]. The basement membrane is an essential element in the diagnosis of any pathological conditions like malignant epithelial tumours. This is because the epithelial cells which are attached to the basement membrane, if gets transformed into cancerous cells and become 'malignant', they bring about a structural deformity in the BM- a break through the basement membrane is observed. A result of which is the invasion of these cancerous cells into the tissues beneath[7].

Oral Squamous Cell Carcinoma (OSCC) is a common malignant tumour that usually affects the oral cavity. It is considered to be one of the most common cancers affecting the oral cavity[8]. As the name suggests it is a carcinoma that develops from the squamous cells of the oral mucosa. It usually affects elderly individuals with a higher frequency observed in males that females[8].The etiology of OSCC is usually multifactorial and is mostly lifestyle associated, mostly habits such as smoking or chewing tobacco with chronic alcoholism[8,9,10]. Other causes include prolonged exposure to ultraviolet (UV) radiation, infective agents, immune defects and genetic mutations which affects the DNA-repair enzymes[8,10,11]. The most common sites of OSCC the lower lip and the lateral part of the tongue. It usually appears as a white, red, or mixed white and red lump or ulcer[8].

Usually the treatment for OSCC is surgery followed by radiation therapy and chemotherapy[8].Though a trained diagnostician can diagnose OSCC from the history and clinical examination, this diagnosis must always be confirmed histologically with repeated biopsies if the clinical picture is consistent with OSCC[8].This diagnosis can be achieved by observing the histopathological features of OSCC as it is the gold standard in the diagnosis of oral squamous cell carcinoma[12].

Oral squamous cell carcinoma, being an epithelial malignancy, is characterized by abnormal cellular division[12]. This abnormal cellular division causes a breachment or a break in the basement membrane, hence allowing the malignant epithelial cells to invade into the underlying connective tissue along with aberrant keratinization in the form of keratin pearls[10,12]. Among all the above, we observed the breachment of basement membrane as it is considered to be one of characteristic features of OSCC.

Hence to observe such changes, staining of the smears of the suspected tissues is a must. For several years, pathologists have been using Hematoxylin and Eosin (H-E) stains[13]. It is popularly used by histotechnologists and pathologists looking at biopsies, surgical and post mortem specimens.But this stain has got its limitations which tends to compromise with the tissue based diagnosis13.

Although the presence of the BM can be detected very specifically by immunohistochemical methods on paraffin-embedded tissue sections, the antikeratin-antisera are not economical and are time consuming. Hence in order to overcome such limitations, special stains were introduced. The “special stain” terminology is most commonly used in the clinical environment and it refers to any technique other than the H-E method that is used to impart colours to a specimen. And one such special stain is PAS (Periodic Acid Schiff).

Hence, this study was designed to compare the efficacy of H-E and periodic acid Schiff (PAS) stain to identify the breachment of basement membrane of SCC under light microscope.

Materials and Method:

This entire study was conducted in Saveetha Dental College and Hospitals, Department of Oral Pathology.Five OSCC tissue samples were collected from the archives of Saveetha Dental College and Hospitals, sectioned by a microtome and two set of slides were prepared for each tissue sample.Then the first set of slides were stained with H and E and the other set of smears were stained with PAS following the staining protocol.

Protocol For H&E Staining[14,15]:

- ✓ Deparaffin and rehydrate slides.
- ✓ Slightly overstain the sections with heamtoxylin 3-5 minutes.
- ✓ Remove excess stain in tap water for 2 minutes.
- ✓ Differentiate and restain a few seconds in acidic alcohol until sections look red.
- ✓ Rinse in tap water.
- ✓ Dehydrate and clear, coverslip with Cytoseal.

Reagents for H&E Staining[14,15]:

- ✓ Acid Ethanol: 1 ml concentrated HCl + 400 ml 70% ethanol
- ✓ Hematoxylin: Harris hematoxylin with glacial acetic acid
- ✓ Eosin: Eosin Phloxine stain
- ✓ Permount: Histological mounting medium

Protocol for Pas Staining[15]:

- ✓ Deparaffinize and hydrate to water.
- ✓ Oxidize in 0.5% periodic acid solution for 5 minutes.
- ✓ Rinse in distilled water.
- ✓ Place in Schiff reagent for 15 minutes (Sections become light pink color during this step).
- ✓ Wash in lukewarm tap water for 5 minutes (Immediately sections turn dark pink color).
- ✓ Counterstain in Mayer's hematoxylin for 1 minute**.**
- ✓ Wash in tap water for 5 minutes.
- ✓ Dehydrate and coverslip using a synthetic mounting medium.

Reagents for Pas Staining[15]:

0.5% Periodic Acid Solution: Periodic acid 0.5 g, Distilled water 100 ml.

Schiff Reagent: Test for Schiff reagent: Pour 10 ml of 37% formalin into a watch glass. To this add a few drops of the Schiff reagent to be tested. A good Schiff reagent will rapidly turn a red-purple colour. A deteriorating schiff reagent will give a delayed reaction and the colour produced will be a deep blue-purple.

All the sets were finally observed under the light microscope under 40x and 100x magnification power, the changes were evaluated and the results were tabulated separately for both the stains.

Results:

The changes in the basement membrane continuity observed under the light microscope under different magnification powers were tabulated considering the following parameters9:

- ✓ Continuity- Continuous or Fragmented
- ✓ Contrast- Distinct or Indistinct

- ✓ Type- Fibrillar or Afibrillar

Frequency and percentage was calculated seperately for the data obtained[9].

In this study, both the staining procedures, i.e. H-E and PAS stains showed a clear BM in pink and magenta pink respectively. In context to H and E, 75% of the cases showed a fragmented, distinct, fibrillar BM while the remaining 25% of the cases showed acontinuos, indistinct, afibrillar BM. When using the PAS stain 75% cases, in context to continuity of the BM, showed a fragmented BM while the remaining 25% of the cases showed a continuous BM. But with regards to the contrast and type of the BM, 50% showed distinct, 50% showed indistinct and 50% showed fibrillar and 50% showed afibrillar type of BM.

Discussion:

As already mentioned above, the basement membrane of the oral mucosa is an important diagnostic element in identifying any pathological conditions like malignant epithelial tumours.

After diagnosis comes treatment planning. The treatment modalities for OSCC depends on the stage at which it is diagnostic which in turn will determine the survival rate of the patient. Hence for such purposes, appropriate stains which are capable of providing finer details with clarity is required. Hence we compared the regular histological stain H and E with a special stain – PAS.

From the present study, it was observed that PAS is better in demonstrating the contrast and pattern of the basement membrane in OSCC tissue specimens when compared to H and E stain. But there was no difference between PAS and H and E in demonstrating the continuity of basement membrane. A similar previous study demonstrated by Ashwini Pujar. et. al concluded that the continuity and contrast along with the homogenous pattern and afibrillar pattern of BM was better demonstrated by acriflavine followed by the PAS stain in Lichen Planus[13]. Though the continuity of the basement is demonstrated equally by H and E and PAS, demonstration of the continuity of the basement membrane with better contrast makes it a better stain than the H and E in identifying OSCC.

Conclusion:

From our study, we found that PAS is better in demonstrating the contrast and pattern of the basement membrane in histopathological specimen like Squamous Cell Carcinoma (SCC) when compared to H and E stain.

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