Original Article

Efficacy of Various Stains to Study Mitotic Figures in Oral Epithelial Dysplasia - A Pilot Study

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Abstract :

Background: Although Haematoxylin and Eosin is a widely used stain to study oral epithelial dysplasia, sometimes studying mitotic figures in sections stained with these stains might pose problems. Mitotic figures is one of the major criterias to assess dysplasia. Various methods to illustrate mitotic figures have been developed over the years including microscopy, morphometry, flow cytometry, nucleotide radiolabelling and immunohistochemistry. But these methods are not cost effective and less feasible for routine use. Selective stains such as crystal violet, giemsa, toluidine blue, feulgen have been used for staining mitotic figures in tissues. ***Aim:*** The present study aims to evaluate effectiveness of various stains for studying mitotic figures. ***Objectives:*** To study the efficacy of various specific stains to highlight mitotic figures in oral epithelial dysplasia. ***Material & methods:*** The study sample includes sections from tissues embedded in paraffin blocks diagnosed as oral epithelial dysplasia. These sections will be stained with various stains and the mitotic figures will be assessed. ***Results:*** Mitotic figures were enhanced with feulgen stain. ***Conclusion:*** The study will be further continued with greater sample size for quantification of mitotic figures with H & E and Feulgen stain.

Keywords: Oral Epithelial Dysplasia; Mitotic Figures; H&E Stain; Toluidine Blue Stain; Giemsa Stain; Crystal Violet Stain, Feulgen Stain

Introduction:

Currently, prognostic evaluation and treatment planning for oral epithelial dysplasia is mainly based on histological grading. Progression of precancer to cancer is believed to be associated with dysplastic features in the epithelium. During development of cancer, the combination of genetic and epigenetic alterations takes place which are reflected by clinical as well as histological changes. The oral precancerous and cancerous lesions can show various clinical presentations. To obtain a confirmatory diagnosis, it is necessary to perform a biopsy of the suspected lesion and study it microscopically.

Dysplasia is a diagnosis defined by the presence of certain histological and cytological features. Increased and abnormal mitosis is considered as one of the significant findings in dysplasia.[1] Mitosis is the process wherein there is equal partitioning of replicated chromosomes and their genes into two identical groups. The process of cell division includes division of nucleus as well as cytoplasm which results in formation of two daughter cells.[2] Mitosis are sometimes distinctly abnormal, that it appears as anarchic multiple spindles in tripolar or quadripolar forms.[3]

To identify the mitotic figures, various stains have been used. Literature search revealed that numerous selective stains such as crystal violet, malachite green with crystal violet, toluidine blue, giemsa and feulgen have been used for staining mitotic figures in tissues.[4]

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Aim : To study the efficacy of various stains to identify the mitotic figures in oral epithelial dysplasia

Objective: Comparision of various stains in highlighting mitotic figures in oral epithelial dysplasia.

Materials and Methods:

The study sample included tissues embedded in paraffin blocks and previously diagnosed as oral epithelial dysplasia, received from the archives of the Dept. of Oral and Maxillofacial Pathology, Sinhgad Dental College and Hospital, Pune. 20 paraffin embedded blocks of tissues were obtained. 5 sections of 3mm each were made from each block and stained with Hematoxylin & Eosin, Giemsa, Crystal violet, Toluidine blue and Feulgen respectively (Fig. 1-5). The study was approved by Institutional Review Board (IRB). The slides were then observed under the Leica Research microscope Model no DM: 1000 with Leica application suite, image analysis software version 4.0 for the mitotic figures under various magnifications.

Mitotic figures were identified by criteria given by Van diest et al

a) The nuclear membrane must be absent indicating the cells have passed the Prophase
b) Clear, hairy extension of nuclear material must be present either clotted (beginning Metaphase), in a plane (Metaphase/ Anaphase), or in a separate clots (Telophase).
c) Two separated parallel chromosome clots to be counted individually as if they are separate mitosis.[5]

Each slide was then observed by separate observers without any exchange of information. Observation made by each observer regarding quality of staining and clarity of mitotic figures were obtained separately.

Results:

All the stains used were nuclear stains but mitotic figures

were enhanced with Giemsa, Crystal violet and Feulgen stain as compared to others. Following table illustrates a comparison between the various stains based on the below mentioned parameters.

Stains	Standar-dization procedure	Time required for staining	Feasibility for routinely use	Quality of mitotic figures seen	Cost effecti-veness
H & E	Easy	20 min	Yes	Good	Economical
Toluidine Blue	Easy	15 min	Yes	Average	Economical
Giemsa	Moderately easy	Overnight	Not frequently	Good	Economical
Crystal violet	Easy	3 min	Yes	Good	Economical
Feulgen	Moderately easy	15 min	Yes	Best	Less economical

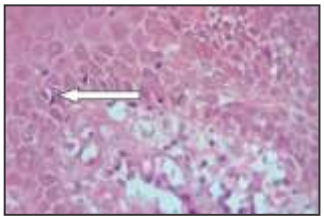

Fig 1. Photomicrographs of H & E stain showing mitotic figures under x40.

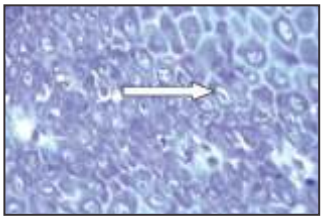

Fig 2. Photomicrographs of Toluidine blue stain showing mitotic figures under x40.

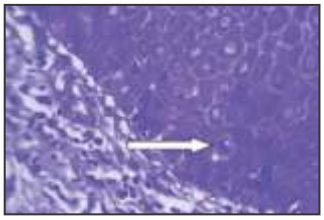

Fig 3. Photomicrographs of Crystal violet stain showing mitotic figures under x 40.

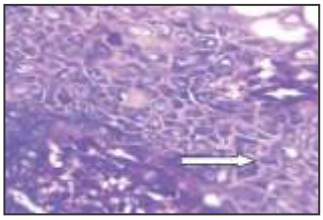

Fig 4. Photomicrographs of Giemsa stain showing mitotic figures under x40.

Discussion:

Mitotic figures is one of the major criterias to assess the dysplasia.[6] Although the new methods to diagnose dysplasia are more specific, time and cost factor makes them less feasible for routine use. A properly standardized histochemical stain and precise use of morphological criterias for identification of mitotic cell can overcome these problems.[7]

The hematoxylin and eosin is the most widely used histological stain. This is because H& E stain shows most histological structures and is particularly suitable for demonstration of nuclei which are the most important structures in every tissue. H&E stain is most readily available but the distinction between an apoptotic cell, a pyknotic nucleus and a mitotic cell sometimes may be difficult.[8] When not sufficient by themselves; they usually provide information to indicate which other staining method is to be used.[9]

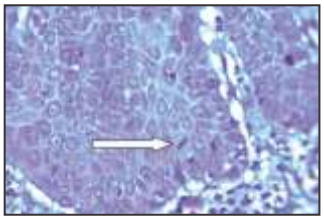

Fig 5. Photomicrographs of Feulgen stain showing mitotic figures under x 40.

Toluidine blue is frequently used adjunctive diagnostic technique to assess oral mucosal disorders.[10]

Over the last 3 decades toluidine blue is used as a vital stain for mucosal lesions and also has found application in staining tissue sections to specifically stain nuclear components because of its metachromatic property.[11] Its main characteristic is that, it selectively stains acidic tissue components.

Therefore, it can also be used for staining mitotic figures.[12] Though it is simple and cost effective and quick to perform, differentiation of mitotic figures from rest of cells was not clear in our study.

Use of acid Giemsa technique for rapid identification of mitotic cells was mentioned by William Doodley. The description of mitotic cells and the classification into Prophase, Metaphase, Anaphase and Telophase can be readily accomplished at magnifications as low as 50-100X.[13] We followed the Bancroft's staining technique for this stain and found that mitosis appeared dark blue and could be easily differentiated from the light pink background. But procedure takes longer time (overnight) and probably not suitable in routine practice.

Crystal violet stain is a step ahead of the standard H&E stain, which facilitates the identification of mitotic figures even at lower magnification as compared to H&E stain.[4] In our study also we found that, staining with crystal violet is quicker, cheaper and comparatively easier to perform.

Feulgen reaction along with micro-spectrophotometry was first employed for evaluation of oral cancer by Doyle and Mantoid in 1975 for predicting the transformation of oral leukoplakia to oral squamous cell carcinoma (OSCC).[14] Relatively few studies have employed feulgen staining for paraffin embedded sections of oral dysplasia and OSCC. Feulgen stained malignant cells display an elevation in nuclear area corresponding to the abnormality in the DNA profiles that is not always evident in PAP stained smears.[15] As Feulgen stain has high DNA specificity, we found a good contrast in staining and mitotic figures could be appreciated even at low magnification.

Conclusion:

The selective stains are a step ahead of H&E stain in staining mitotic figures. These stains can also be used as an adjunct to

study dysplasia. Although in our study we found all the stains useful in identification of mitotic figures, feulgen stains gave us the best result. The study will be further continued with greater sample size for quantification of mitotic figures with H & E and Feulgen stain.

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How to cite this Article: Palaskar S, Patil S, Narang B, Prabhu P, Kathuriya P, Pawar R. Efficacy of various stains to study mitotic figures in oral epithelial dysplasia -A pilot study. J Dent Allied Sci 2013;2(2):46-48.
Source of Support: Nil. **Conflict of Interest:** None Declared.