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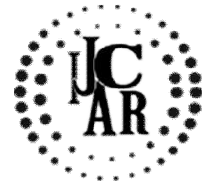

Research Article

COMPARISON OF MITOTIC FIGURES BY DIFFERENT STAINS IN ORAL EPITHELIAL DYSPLASIA AND SQUAMOUS CELL CARCINOMA

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A B S T R A C T

Mitotic figures are one of the major criteria 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. The present study aims to evaluate effectiveness of various stains for studying mitotic figures and comparison of mitotic figures in oral epithelial dysplasia and oral squamous cell carcinoma. **Material & methods:** The study sample includes sections from tissues embedded in paraffin blocks diagnosed as oral epithelial dysplasia and oral squamous cell carcinoma. These sections will be stained with various stains and the mitotic figures will be assessed. **Results:** Mitotic figures were enhanced with feulgen stain. Although no statistically significant results were obtained on comparing the study groups. **Conclusion:** 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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INTRODUCTION

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 of cells gives rise to tissue integrity. Excessive proliferation of cells due to increased mitosis is one such outcome, which is the hallmark in precancer and cancer.[(2)]. Errors in identifying a mitotic cell can weaken the reliability of histological grading due to the loose use of morphologic criteria. Combination of stains and modification of the existing histochemical techniques can overcome these problems. 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.[(3)]

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Hence the present study aims to study the efficacy and compare various stains to identify the mitotic figures in normal oral mucosa, epithelial dysplasia and oral squamous cell carcinoma.

MATERIALS AND METHODS

Ten tissues of normal oral mucosa and 15 tissues each of oral epithelial dysplasia seen in tobacco-associated leukoplakia and squamous cell carcinoma were retrieved from the archives of the Department. Two sections of 3 microns each were cut from the blocks and stained with Hematoxylin & Eosin, Giemsa, Crystal violet, Toluidine blue and Feulgen respectively.

Mitotic figures were counted using an oculometer grid in 15 grid fields under a magnification of x400 in a stepladder fashion. The oculometer grid had 10 x 10 squares. But, the periphery of the square grid was restricted by the limited, circular field of the eyepiece. So, only the central, 4 columns and 10 rows (4 x 10 = 40 squares) were considered for the purpose. Hence, a grid field in this study consisted of 40 squares.

Criteria to identify the mitotic cells

The criteria given by Van Deist *et al*. [6] were used to assign a structure as a mitotic figure in this study:

1. The nuclear membrane must be absent indicating that cells have passed the prophase.

2. Clear, hairy extension of nuclear material must be present either clotted (beginning Metaphase), in a plane (Metaphase/ Anaphase), or in a separate clots (Telophase).
3. Two separated parallel chromosome clots to be counted individually as if they are separate mitosis. [(4)]

Mitotic count was expressed as the mitotic count per grid field and the mitotic count per square millimeter.

The mitotic count per grid field was calculated as,

$$\text{mitotic count / grid field} = \frac{\text{Total number of mitotic figures observed}}{\text{Number of grid fields counted}}$$

The mitotic count per square milimeter was calculated as follows:

a. Area of 1 grid field = 0.0128 mm^2
b. Mitotic count per square milimeter =Average number of mitotic figures per grid field

$$\frac{\text{Average number of mitotic figures per grid field}}{0.0128\ mm^2}$$

Collected data was statistically analyzed using the Mann-Whitney U test at a confidence interval of 95%. All the data analysis was done using the Statistical Package for Social Sciences (SPSS) [Version 10].

RESULTS

All the stains used were nuclear stains but mitotic figures were enhanced with Giemsa, Crystal violet and Feulgen stain as compared to others.

Amongst all stains used, we found a good contrast in staining and mitotic figures could be appreciated with Feulgen stain even at low magnification.

Table 1

Stains	Efficacy	Time required	Cost effectiveness
H & E	Good	20 min	Economical
Toluidine Blue	Average	15 min	Economical
Giemsa	Good	Overnight	Economical
Crystal violet	Good	3 min	Economical
Feulgen	Best	15 min	Less Economical

No mitotic cells were seen either in ten cases of normal oral mucosa. In epithelial dysplasia, the mean mitotic count /15 grid fields was 4.566 (SD = standard deviation =3.896).
In carcinoma group, mean mitotic count of 8.966/15 grid fields was obtained (SD = standard deviation =8.665).

Table 2

	Mean number of mitotic figures/grid field	Statistical significance
Oral epithelial dysplasia	4.566	u-value = 0.6642 P value = 0.4560 Not Significant
Oral squamous cell carcinoma	8.966	

No significant results were obtained when comparison of mitotic counts was made between the dysplasia and the carcinoma groups.

DISCUSSION

Mitosis, the cell generating process in humans, runs at the nuclei level and, as such, there is an interest in studying the nuclei of cells with the purpose of detecting cancerous cells.[5]Thus identification and quantitation of mitotic cells forms an indivisible part of the histological grading systems used for prognostication of precancerous and cancerous lesions. The quantitation of mitotic figures has been on the backseat over the decades. Newer prognosticators like immunohistochemistry, flow cytometry, autoradiography, DNA ploidy measurements are now on the forefront.

However, cost and time factors make them less feasible. A properly standardized histochemical stain and precise use of morphologic criteria for identification of a mitotic cell can overcome these problems.

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.[6] When not sufficient by themselves; they usually provide information to indicate which other staining method is to be used.[7]

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.[8] Its main characteristic is that, it selectively stains acidic tissue components. Therefore, it can also be used for staining mitotic figures.[9] 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. 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.[3]

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.[8] As Feulgen stain has high DNA specificity, we found a good contrast in staining and mitotic figures could be appreciated even at low magnification.

Increases in mitotic index in dysplasia and carcinoma as compared to normal oral mucosa indicate an important kinetic change, possibly representing increased stem cell turnover.[9] Also volume corrected mitotic index (mitotic figures per square mm of neoplastic tissue in the microscopic field) is considered as the best predictor of prognosis.[10]

The mitotic cells were numerous in basal and parabasal cell layers along with suprabasal layers in oral epithelial dysplasia whereas mitotic figures were distributed all throughout the superficial epithelium as well as nests, cords and sheets of infiltrating epithelial cells in squamous cell carcinoma. Also, in neoplastic tissues, the number of prophases is greatly reduced relative to metaphase stage which is of considerably longer duration. The relative number of anaphase stages shows

also a tendency to decrease. This could be attributed to the fact that in cancer cells the relative duration of prophase stages is greatly reduced which would mean that the formation of the spindle is more rapid. The abnormal mitotic figures are due to an abnormal increase in the number of centrioles or to a lack of coordination between the chromosomal cycle and the division of centrioles.[3,11]

Therefore, considering all these drawbacks, although notoriously considered unreliable, mitotic cell counting is the easiest, cheapest and fastest way of assessing proliferation.It can be reproducible when precisely standardized staining techniques and identification criteria are strictly followed. Thus, the present study recommends the use of Fuelgen stain as a selective stain for mitotic figures.

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. How far a single tissue section can be representative of an entire tumor mass is a matter of debate. Thus, it is recommended that larger samples of the study groups and serial sections should be ideally studied to determine the reliability of mitotic figure counting as a reliable parameter.

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