Comparison and Evaluation of Mitotic Figures in Oral Epithelial Dysplasia using H & E and Toluidine blue stains

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Abstract:-

*Background:-*Mitotic figures refer to the various phases of mitosis which are well appreciated in tissue sections.They act as prognostic indicators in oral epithelial dysplasia.

*Aim:-*To compare and evaluate the mitotic figures in oral epithelial dysplasia using H & E and Toluidine blue stains.

*Objective:-*Using H&E, Toluidine blue stains in the identification and counting of mitotic figures in diagnosed cases of oral epithelial dysplasia and thereby to evaluate their efficacy.

*Materials and 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 Toluidine blue stain.

Keywords:- Oral epithelial dysplasia, Mitotic figures, H&E stain, Toluidine blue stain.

INTRODUCTION:-

Oral potentially malignant disorders diagnosed as Oral epithelial dysplasia are significantly associated with increased risk of cancer[1]. Progression of pre-cancer to cancer is associated with features of dysplasia in the epithelium. Mitosis is the process where there is equal partitioning of replicated chromosomes and their genes into two identical groups and serves as the basis for cell proliferation. Mitotic figures refer to the various phases of mitosis (prophase, metaphase, anaphase, telophase) which are well appreciated in tissue sections[2,3]. They are defined as atypical if they display excessive no.of mitotic spindles with a multipolar morphologic appearance. They act as prognostic indicators in oral epithelial dysplasia[4]. Advanced techniques like flow cytometry, IHC, auto radiography have been used previously to detect mitotic figures but they are expensive and time consuming and so selective stains like Toluidine blue, Giemsa, Crystal violet, Feulgen stain have been used to stain mitotic figures in tissues[3,5].

Hematoxylin and Eosin (H & E) is a routinely used histological stain used in pathology to get a detailed view of the tissues. It clearly stains the cell structures like nucleus, cytoplasm, cell organelles and extra-cellular components. This usually gives sufficient information to diagnose diseases based on the arrangement and appearance of cells and abnormalities which act as indicators of a disease[1].

Vital staining is the staining of cells or tissues in the living state. Toluidine blue is a vital acidophilic metachromatic die that stains acidic tissue components. It has an affinity for nucleic acids. It highlights the potentially malignant oral lesions which could be missed out on clinical examination. It is useful in selecting the biopsy sample site in premalignant lesions. TB-stained tissue may appear dark royal blue or pale royal blue colour[6]. Connective tissue mucins stain purple to red while the backgrounds stained blue with TB. When 1% TB is used *Helicobacter* stains dark blue. Endocrine cell granules are also stained purple to red. Mast cell granules purple[6].

MATERIALS AND METHODS:-

The study sample included tissues embedded in paraffin blocks and previously diagnosed as oral epithelial dysplasia, retrieved from the archives of the Department of Oral Pathology, Saveetha Dental College and Hospitals, Chennai. 6 paraffin embedded blocks of tissues were obtained. 2 sections of 3μm each were made from each block and stained with H & E, Toluidine blue. Each slide was then observed by separate observers under a microscope without any exchange of information. Observation was made by each observer regarding the intensity of staining, discernibility and the no.of mitotic figures.

Mitotic figures were identified by the criteria given by Van Diest et.al.

1) The nuclear membrane must be absent indicating the cells have passed the prophase.
2) Clear, hairy extension of nuclear material (condensed chromosome) must be present either clotted (beginning metaphase), in a plane (metaphase/anaphase) or in separate clots (telophase).
3) Two parallel, clearly separate chromosome clots to be counted individually as if they are separate mitoses[1,2,3,4].

RESULTS:-

The interclass correlation coefficient for the no.of mitotic figures in H & E stain between observer 1 and observer 2 was 0.997. The interclass correlation coefficient for the no.of mitotic figures in Toluidine blue stain between observer 1 and observer 2 was 0.999. There is good interobserver agreement between observer 1 and observer 2 in relation to the no.of mitotic figures observed in H&E and Toluidine blue stains separately. The values are statistically significant (p=.000).

Correlations

		H & E stain	Toluidine blue stain
H & E stain	Pearson Correlation	1	.998[**]
	Sig. (2-tailed)		.000
	N	6	6
Toluidine blue stain	Pearson Correlation	.998[**]	1
	Sig. (2-tailed)	.000	
	N	6	6
[**]. Correlation is significant at the 0.01 level (2-tailed).			

Scale Statistics

Mean	Variance	Std. Deviation	N of Items
65.00	4941.600	70.297	2

Intraclass Correlation Coefficient

	Intraclass Correlation[a]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.999[b]	.990	1.000	1372.667	5	5	.000
Average Measures	.999[c]	.995	1.000	1372.667	5	5	.000
Two-way mixed effects model where people effects are random and measures effects are fixed.							
a. Type C intraclass correlation coefficients using a consistency definition-the between-measure variance is excluded from the denominator variance.							
b. The estimator is the same, whether the interaction effect is present or not.							
c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.							

Scale Statistics

Mean	Variance	Std. Deviation	N of Items
67.67	5164.267	71.863	2

Intraclass Correlation Coefficient

|	Intraclass Correlation[a]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.997[b]	.978	1.000	645.533	5	5	.000
Average Measures	.998[c]	.989	1.000	645.533	5	5	.000
Two-way mixed effects model where people effects are random and measures effects are fixed.							
a. Type C intraclass correlation coefficients using a consistency definition-the between-measure variance is excluded from the denominator variance.							
b. The estimator is the same, whether the interaction effect is present or not.							
c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.							
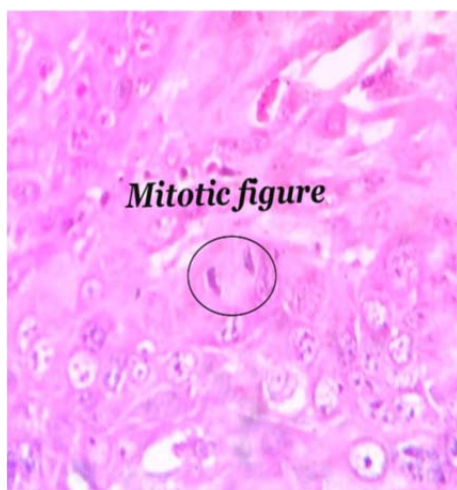
H & E stain
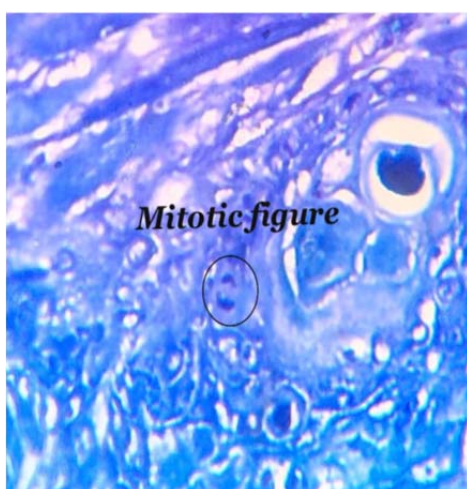
Toluidine blue stain

DISCUSSION:-

Dysplasia is associated with altered tissue architecture including cellular proliferation leading to the malignant transformation if left untreated[5]. Mitotic figures is one of the reliable criteria to assess dysplasia. Mitotic figures should be carefully evaluated to assess cellular proliferation. H & E stain and Toluidine blue stains have been used over decades to stain mucosal lesions. H & E is the most widely used histologic stain due to its easy availability. It shows most of the histologic structures but sometimes differentiation of mitotic figures maybe difficult. This is when alternate special stains like Toluidine blue are indicated. Toluidine blue has metachromatic property and stains acidic components and hence it has been used to stain mitotic figures. It is based on the fact that dysplastic cells may contain more nucleic acids than normal cells. The high density of nuclear material, loss of cohesion and increased mitosis maybe the reason for the easy uptake of Toluidine blue stain by the dysplastic cells[6]. Lingen et al, in their review mentioned the sensitivity and

specificity of TB in the detection of oral cancer to be in the range of 78-100% and 31-100%, respectively[7]. Epstein et al showed sensitivity and specificity of 92.5% and 63.2%, respectively in their results[8]. Another study found 100% sensitivity and 52% specificity[9]. Onofre et al evaluated the TB staining in pre malignancies and superficial oral ulceration suggesting malignancy and 100% sensitivity with no false results[10]. Hedge et al found a sensitivity of 97.29% and specificity of 62.5%[11]. Gupta et al demonstrated sensitivity of 96.9% and specificity of 86% for detection of malignancy using TB[12]. The results of our study in relation to the no.of mitotic figures observed was in accordance with the previous studies conducted.

CONCLUSION:-

H & E and Toluidine blue stains stain the dysplastic tissues. Being a metachromatic, acidophilic vital stain, the mitotic figures are enhanced with the use of Toluidine blue stain.

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