



## USE OF CRYSTAL VIOLET STAIN FOR THE ASSESSMENT OF MITOTIC FIGURES IN ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

Dr. Bipin Gandhi

Associate Professor Department of Pathology Vedanta Institute of Medical Sciences Dahanu, Palghar, Maharashtra.

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**Address for Correspondence:** Dr. Bipin Gandhi, Associate Professor Dept. of Pathology, Vedanta Institute of Medical Sciences Dahanu, Palghar, Maharashtra.

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### Abstract

**INTRODUCTION:** Histopathological grading depends on several features, of which appearance of mitotic figures and apoptotic bodies in the tissue sections plays an important role. The mitotic figures and apoptotic bodies help assess the cellular proliferation and the turnover of the tumor. As mitotic figures (MF) and apoptotic bodies are difficult to assess under routine H and E sections, other methods such as immunohistochemistry (IHC) and flow cytometry have been implemented but, these methods are expensive and more time-consuming and are highly technique sensitive. Hence, stains, which should be cheap, and can be used routinely, and can be tried in identifying the mitotic figures and apoptotic bodies, which has come out with a better result. Routine histological procedures cannot differentiate between pyknotic *nuclei*, apoptotic cells and MFs which makes the diagnosis difficult.

**MATERIAL AND METHODS:** Study samples were divided in to 3 groups, group 1- case group in which 40 samples of paraffin blocks diagnosed as oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) and group 2- control group in which 40 samples from the healthy individuals. 5 micron thickness sections were made from each tissue specimen. One section was stained with H & E stain and another section was stained with 1% crystal violet stain.

**RESULTS:** In case group there were 24(60%) male and 16(40%) female while in control group there were 22 (55%) male and 18(45%) female. Mean age in case group was  $36.2 \pm 15.4$  and in control group was  $25.7 \pm 12.7$ . There is a significant increase in mitotic figures of H & E ( $P < 0.01$ ) and crystal violet ( $P < 0.01$ ). In case, there was a significant ( $P < 0.05$ ) increase in mitotic figures when stained with crystal violet. As compared to H&E stain there was a significant ( $P < 0.01$ ) increase in metaphase observed in crystal violet stain. Mean mitotic figure by H&E in case group was  $4.15 \pm 1.07$  while in crystal violet it was  $5.97 \pm 1.87$ .

**CONCLUSION:** % crystal violet stain is the cheaper stain and can be performed in small laboratories and resource constraint settings. Crystal violet can be used for the localization of mitotic figures and assessing proliferation.

## Introduction:

Oral squamous cell carcinoma is one of the most common cancers of the oral cavity. The grading of the lesion will determine the treatment and prognosis of this cancer. However, the grading of oral squamous cell carcinoma has been a subjective phenomenon, and it depends on the pathologist's perspective. Histopathological grading depends on several features, of which appearance of mitotic figures and apoptotic bodies in the tissue sections plays an important role. The mitotic figures and apoptotic bodies help assess the cellular proliferation and the turnover of the tumour<sup>i</sup>. Development of cancer is from the series of uncontrolled cellular events known as *atypia* in which cellular and nuclear morphometric changes occur because of the result of excessive alterations in DNA synthesis accompanied by proliferation and *apoptosis*<sup>ii</sup>. Apoptosis can be defined as programmed cell death which occurs normally as a part of organism's growth. Apoptotic cells are identified by their reduced size and the presence of highly condensed chromatin<sup>iii</sup>. There is a considerable variation in cancer cells in terms of morphology and dimensions, which includes pleomorphism and increase in increased nucleus-cytoplasmic ratio<sup>iv</sup>

As mitotic figures (MF) and apoptotic bodies are difficult to assess under routine H and E sections, other methods such as immunohistochemistry (IHC) and flow cytometry have been implemented but, these methods are expensive and more time-consuming and are highly technique sensitive. Hence, stains, which should be cheap, and can be used routinely, and can be tried in identifying the mitotic figures and apoptotic bodies, which has come out with a better result. Routine histological procedures cannot differentiate between pyknotic *nuclei*, apoptotic cells and MFs which makes the diagnosis difficult<sup>v</sup>.

In the resource limited settings stains such as Feulgen and 1% crystal violet stains which depend on the acid hydrolysis of DNA can be used to demonstrate chromosomal material in

cells and tissues<sup>vi</sup>. This study was carried out with the aim to evaluate effectiveness of crystal violet stain for MFs and its comparison with routine hematoxylin and eosin (H & E) stain.

## MATERIAL AND METHODS

This is a case control study which was carried out in the department of Pathology at Vedanta Institute of Medical Sciences Dahanu, Palghar, Maharashtra. Study samples were divided into 3 groups, group 1- case group in which 40 samples of paraffin blocks diagnosed as oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) and group 2- control group in which 40 samples from the healthy individuals were taken. Patients with history of diabetes, hypertension, and bleeding disorders were excluded from the study as diabetes mellitus (DM) and hypertension will affect the rate of mitosis through altered metabolism of cell and oxygen supply, respectively<sup>vii</sup>.

Grades of OSCC as well as differentiated (WDSCC), moderately differentiated (MDSCC) and poorly differentiated OSCC (PDSCC), was used. OED was placed into three grades as mild epithelial dysplasia (MIED), moderate epithelial dysplasia (MOED) and severe epithelial dysplasia (SED).

5 micron thickness sections were made from each tissue specimen. One section was stained with H & E, another section was stained with 1% crystal violet stain, both stains were used from Fisher Scientific Company.

Each slide was observed by two different pathologists. Statistical analysis was done by using the Man-Whitney U test. *P*-value of <0.05 was considered as statistically significant. All the data analysis was done using the Statistical Package for Social Sciences (SPSS).

## RESULTS

Total number of study sample was 80, in case group 40 and in control group 40. In case group there were 24(60%) male and 16(40%) female while in control group there were 22 (55%) male

and 18(45%) female. Mean age in case group was 36.2±15.4 and in control group was 25.7±12.7.

**Table 1: group characteristics**

|          | Case Group (n=40) | Control group (n=40) |
|----------|-------------------|----------------------|
| Male     | 24(60%)           | 22 (55%)             |
| Female   | 16(40%)           | 18(45%)              |
| Mean age | 36.2±15.4         | 25.7±12.7            |

**Table 2: Table 1: Distribution of mitotic figures in two with different phases of mitosis**

| Variable | Group (n=40) | Case mean±SD |
|----------|--------------|--------------|
| H&E MF   | Case         | 4.15±1.07    |
|          | Control      | 0.92±0.70    |
| CV MF    | Case         | 5.97±1.87    |
|          | Control      | 1.24±0.57    |
| H&E-M    | Case         | 3.54±1.04    |
|          | Control      | 0.81±0.74    |
| CV- M    | Case         | 5.24±1.98    |
|          | Control      | 1.41±0.80    |
| H&E-A    | Case         | 0.81±0.3     |
|          | Control      | 0.34±0.21    |
| CV- A    | Case         | 1.42±0.91    |
|          | Control      | 0.28±0.34    |
| H&E-T    | Case         | 0.33±0.48    |
|          | Control      | 0.00±0.00    |
| CV- T    | Case         | 0.24±0.29    |
|          | Control      | 0.00±0.00    |

MF: mitotic figure, CV: crystal violet, H&E: Hematoxin and eosin, M: Metaphase, A: Anaphase, T: Telophase, SD: Standard deviation. There is a significant increase in mitotic figures of H & E ( $P < 0.01$ ) and crystal violet ( $P < 0.01$ ). In case, there was a significant ( $P < 0.05$ ) increase in mitotic figures when stained with crystal violet. As compared to H&E stain there was a significant ( $P < 0.01$ ) increase in metaphase observed in crystal violet stain.

**DISCUSSION & CONCLUSION**

Around 2-4 % cancer cases around the world are oral cancers. The prevalence of oral cancer is higher, reaching the 10% of all cancers in Pakistan and around 45% in India<sup>viii, ix</sup>. The common risk factor for oral cancer is tobacco<sup>x</sup>. The assessment of mitotic figures is commonly used for making a diagnosis and assessment of

prognosis in oral epithelial dysplasia (OED) and OSCC<sup>xi</sup>. Light microscopy is an indirect measure to assess the significance of apoptosis as a marker in dysplastic lesions and malignant epithelial lesions of the oral cavity<sup>xii</sup>.

Routine H & E stain does not give precise view for identification of the mitotic figures or the apoptotic bodies. So there is a need of special stains, which should be cost-effective, less time-consuming, and less technique sensitive, should be used in enhanced assessment of these mitotic figures and apoptotic bodies.

In our study mean mitotic figure by H&E in case group was 4.15±1.07 while in crystal violet it was 5.97±1.87. This difference was statistically significant. More number of MF in crystal violet stain was observed as crystal violet stain clearly stains the chromosome leaving cytoplasm clear and unstained due to hydrolysis of tissue sections by HCl at 60°C which help in easy identification of MF and also offer a more reliable counting. Also crystal violet staining provides identification of mitotic figures at a lower magnification as compared to H & E stained section. In a study by Jadhav *et al*<sup>xiii</sup>. and Tandon *et al*<sup>xiv</sup> used crystal violet to measure MI where crystal violet is proved to be a better stain over H & E. Rao *et al*. in their study compared the three stains and concluded that Feulgen was the best stain for mitotic figures<sup>xv</sup>. Chieco *et al*<sup>xvi</sup> used nuclear fast red as counter stain with 1% crystal violet, and concluded that mitotic figures stands clearly against red background.

To conclude 1% crystal violet stain is the cheaper, & most feasible staining techniques and can be performed in small laboratories and resource constraint settings. Crystal violet can be used for the localization of mitotic figures and assessing proliferation. So 1% crystal violet stain can be used in daily practice for identification of mitotic figures.

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