

# Effectiveness of crystal violet stain for localization of mitotic activity in oral squamous cell carcinoma

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## ABSTRACT

**Background:** Mitotic figure counting is simplest and oldest method for determining proliferative activity of cell. It is considered as one of the important diagnostic aid in cancer pathology. Though advanced methods to evaluate dysplastic features are more precise and definite but expense and time makes them less practicable for routine use. Therefore an effort was made to use economical as well as simple approach involving crystal violet stain (1%) to study the mitotic figures in oral squamous cell carcinoma.

**Materials and methods:** This descriptive research included samples, consisting of thirty three cases of the oral squamous cell carcinoma (OSCC). Representative sections were stained with H&E stain and 1% crystal violet stain respectively. The stained sections were viewed under optical microscope to count mitotic figures for evaluating the effectiveness of 1% crystal violet stain. Data obtained was statistically analyzed by using sample t-test.

**Results:** There was noteworthy increase in the mean mitotic count among the crystal violet stained sections of OSCC in contrast to the OSCC sections stained with H&E (P = 0.00).

**Conclusion:** 1% Crystal violet stain can be considered as one of the optimum stains to observe the mitotic figure. Practice of staining with 1% crystal violet during routine histopathological procedures will be cost effective and may be used as a selective stain.

**Keywords:** Crystal violet stain, oral squamous cell carcinoma, mitotic figures

## INTRODUCTION

The rate of mortality of head and neck cancers remains high with approximately 650, 000 new cases and about 300, 000 deaths occur globally every year. <sup>1,2</sup> Squamous cell carcinoma of oral cavity is one of the ten utmost frequent malignancies in Pakistan, frequently involving the floor of the mouth, tongue and inferior lips. <sup>3, 4</sup>

Unprompted mutation in the DNA is the underlying basis and its prognosis is quiet discouraging with survival rate of 30% at 5 years. <sup>5, 6</sup> The early diagnosis of disease is very significant in improving the survival rate of the patient. <sup>7</sup> Enhanced growth period is the primary effect after exposure to stimuli. Irreversible damage occur when reversible injury stages surpasses resulting in necrosis or progression into malignancy. Dysplastic epithelium is usually the primary microscopic presentation in many cases of cancer. <sup>8</sup>

Mitosis consists of splitting of parent cell into two similar daughter cells. The different phasaes of mitosis are prophase, metaphase, anaphase and telophase. Defects of Mitosis lead to abnormalities at nuclear level like binucleation, pyknotic nuclei, rise in quantity of mitotic figures and atypical mitotic figures. In OSCC, occurrence of atypical mitosis or increase in quantity of mitosis is the significant finding. <sup>9</sup> Mitosis reflects proliferation activity of the cell and serves vital part in describing the severity and prognosis of the condition. <sup>10</sup>

Atypical mitotic activity and increased in their number indicates genetic injury. Therefore, identification and quantification of the mitosis is significant part of different grading system for establishing the prognosis of neoplastic conditions. <sup>9</sup> Several authors described numerous techniques and stains to identify the mitotic activity. <sup>10</sup> Earlier researches have revealed different distinguished stains like giemsa, crystal violet and toluidine blue which can identify chromatin patterns. <sup>11</sup> Among which, Crystal violet based on hydrolysis of DNA is employed to observe the nuclear changes in cells. <sup>12</sup>

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## MATERIALS AND METHODS

This descriptive study was carried out at Histopathology department of the Post Graduate Medical Institute, Lahore and included 33 specimens of OSCC following the inclusion and exclusion criteria. Specimens were collected from OSCC patients of all ages with both genders whereas specimens of OSCC patients with chronic debilitating conditions were excluded from the study. The study was conducted from December, 2015 to December 2016. The representative sections were taken from paraffin-embedded archival tissue blocks and divided further into 2 sections, subjected to stain with H&E and 1% crystal violet respectively. The study protocol was approved by ethical committee of the respective institution. Fraser FJ modified method was used for Crystal Violet staining<sup>13</sup>. The prepared slides were then observed under research microscope (YUIE YJ-121B 1000X Binocular Optical Microscope). Anneroth's multifactorial grading system is used for grading of SCC.<sup>4</sup> The standards specified by Van Deist and his colleagues was applied in this research to identify mitotic figure in order to distinguish among different mitosis phases from frequently appreciated other alterations in nucleus like apoptosis, karyorrhexis and pyknotic nuclei. Mitotic figure counting was done according to the method described by Culling<sup>14, 15</sup>. After placing over the ocular graticule on the slide, counting of mitotic figures was performed. Each slide was then viewed for mitotic figures counting by using ocular grid eyepiece under high power field (400X). 10 different high power fields was observed for counting in stepladder fashion. The area chosen for mitotic figures counting involved the most cellular as well as invasive part of the tissue. The areas excluded for counting were the areas displaying necrosis, tissue folds, inflammation and calcifications. Mitotic cell count was determined as mitoses per mm<sup>2</sup> and the count of mitosis each grid field; Mitotic cell count/Grid field = Total sum of mitotic figures observed/Number of grid fields counted, Mitotic count per square millimeter = Average number of mitotic figures per grid field / 0.25mm<sup>2</sup> where 0.25 mm<sup>2</sup> is the area of one grid field calculated in this study.<sup>15</sup> The result was stated as mitoses per mm<sup>2</sup> that is mitosis in 1 square millimeter of the malignant tissue in the current research. The factors like field diameter, numeric aperture of microscope are hard to monitor, so mitosis for each high power field may show variability and as a result, outcomes of study cannot be evaluated among several laboratories. Hence, counting per square millimeter minimized the necessity for further standards and provided noteworthy outcomes.<sup>13</sup> All slides were

Table 1. Mean mitotic count among OSCC sections stained with H&E and crystal violet

Characteristics	Hematoxylin and eosin	Crystal violet	p-value
Mitotic count in OSCC cases	10.8	12.8	<0.01
Mitoses per mm <sup>2</sup>	4.3	5.1	<0.01

observed by 2 histopathologists separately and data was entered into the Statistical Package for Social Sciences (SPSS) version 20 for analysis. A t-test was used for comparing the effectiveness of staining of mitotic figures between H&E and crystal violet in OSCC. A p-value  $\leq 0.05$  was regarded as being statistically significant.

## RESULTS

OSCC found higher in males (1.75:1) with age age ranged from 22 to 70 years (46.03  $\pm$  11.40) in the current research. Among 33 cases, buccal mucosa (57.6%) was the most commonly affected oral cavity site following dorsal surface of tongue, retromolar area and lip (27.3%, 9.1%, 6.1%) respectively.

Well differentiated squamous cell carcinoma (WDSCC) was the most prevalent histological grade with (n=18, 54.5%) cases followed by moderately differentiated squamous cell carcinoma (MDSCC) (n=14, 42.4%) and poorly differentiated squamous cell carcinoma (PDSCC) (n=1, 3.0%) histological grades respectively.

Average count of mitotic cell among 33 sections of OSCC was 4.3879 for each 10 grid fields in stained sections of H&E and was 5.1515 per 10 grid fields in the crystal violet stained sections (p=0.00). A significantly increased mitotic count was noticed in OSCC crystal violet stained sections in comparison with H and E-stained counterparts (p=0.00) (Table 1).

## DISCUSSION

Mitotic figure counting is simplest and oldest method for determining proliferative activity of cell. It is considered as one of the important diagnostic aid in cancer pathology as proliferation of cell is commonly used for diagnostic purposes and help in assessing the prognosis of tumors.<sup>16</sup>

One of the most important criteria to evaluate the dysplasia is Mitotic figure. Though advanced methods to evaluate dysplastic features are more precise and definite but cost and time makes them less feasible for routine use. These problems can be overcome by use of properly standardized histochemical stain.<sup>17</sup>

The present study is conducted to use economical as well as simple approach to observe the mitotic figures. This research involves application of crystal

violet (1%) as a discriminating dye and then comparing it with routine H&E dyed sections of OSCC. Errors in methodology are of significant concern while evaluation of mitotic figures.<sup>18</sup> Therefore, in this study well standardized method was used for counting of mitotic figures, considering numerous fields of microscope in the representative sections of the tumor. In this analysis, the average mitotic count per 10 grid fields was 4.3879 in 33 stained sections of OSCC by H&E where as it was 5.1515 per 10 grid fields for the sections subjected with crystal violet staining. In the current study, the aim was to define easy, inexpensive and rapid procedure for identification of the mitotic activity as well as to assess their part in grading of OSCC histologically. It was seen that use of 1% Crystal Violet provided significant staining of mitosis in contrast to routine H&E.

The study on the mitotic figures parallel to current study was carried out by Ankle MR. The study aimed to define selectivity of 1% crystal violet stain by relating it with the methodology for H&E staining. The research outcomes revealed raised in average mitotic count among 1 % crystal violet stained sections in contrast to sections dyed with H&E <sup>11</sup>. In the present study, it is easily perceived that crystal violet offers effective dyeing of mitotic figures and support in its detection.

In another research conducted by Jadhav and coauthors , it was noticed that application of 1% crystal violet delivered definite advantage over the stained sections by H&E. A noteworthy rise in mitotic figures number was noted in OSCC.<sup>19</sup> The existing study is in harmony with the earlier done researches.

Because of basic nature of crystal violet, it has high sensitivity for chromatin which is acidic in nature. Hence explaining the reason of higher sensitivity of crystal violet for mitotic figures <sup>11</sup>. Amendment of staining method by the use of 1N HCL at 60 ° C could explain the overall greater diagnostic efficacy of crystal violet and consequently causing rise in contrast because of decreased RNA content after the hydrolysis <sup>13</sup>.

## CONCLUSION

Crystal violet can be considered a reliable method for the localization of mitotic activity during the routine histopathological procedures for selective staining.

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