RESEARCH ARTICLE

COMPARISON OF STAINING OF MITOTIC FIGURES BY H AND E AND CRYSTAL VIOLET STAINS IN ODONTOGENIC TUMORS WITH AGGRESSIVE BEHAVIOUR- A PRELIMINARY STUDY

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ABSTRACT

Background: Some odontogenic tumors are clinically benign but they are locally aggressive and recur. Therefore such lesions should be completely evaluated for their mitotic potential. Routine staining procedures often pose a problem in differentiating mitotic cells. Although various new methods have been recommended for identifying mitotic figures (MFs) in tissues, the time factor and cost makes them less feasible. Thus, an attempt was made to evaluate the efficacy of crystal violet in identifying MFs.

Aim of the Study: 1) To compare various phases & number of mitotic cells in haematoxylin and eosin stain with that in crystal violet stain in odontogenic tumors with aggressive behaviour.

Materials and Methods: This case control study included samples randomly selected from archives comprising of benign odontogenic tumors which behaved aggressively even though they were benign such as CEOC, PIOC, and Ameloblastoma etc. The control group comprised of squamous cell carcinoma & normal epithelium. Both the groups were stained with H and E stain and 1% crystal violet stain. The stained sections were observed under research microscope for identification of various phases of cell cycle and counting of MFs.

Observations and Results: Data obtained was statistically analysed by using the two tail t test.

Conclusion: Crystal violet stain could be considered as best stain to study mitotic figures in lesions with high proliferative potential.

INTRODUCTION

Cell proliferation is an uncontrolled event in various neoplasms due to presence of abnormal & bizarre mitosis. Various genetic alterations take place during cell proliferation. Mitosis is a process where there is equal division of chromosomes and their genes into two identical groups & serves as the basis for cell proliferation (Palaskar et al., 2013).

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Therefore, the study of mitosis is very important to analyse the aggressiveness and prognosis of lesions. There are various stains and techniques available to detect or study mitotic activity which was reported by various authors. Advance techniques such as IHC, flow cytometry, nucleotide radiolabelling, DNA ploidy, autoradiography have been tried in the literature. These methods however are time consuming and expensive. Hence special stains can be used for easy identification of mitotic figures (Jadhav et al., 2012). Routinely used Haematoxylin and Eosin poses a problem as it cannot help to differentiate between mitotic cell and apoptotic cells & does not give the clear picture of the various phases of cell cycle (Ankle et al., 2007). A literature search reveals that various
stains such as crystal violet, malachite green with crystal violet, toluidine blue, giemsa and Feulgen have been used to identify mitotic figures. Amongst which, Crystal violet is one which is used to study the chromosomal pattern in cells based on acid hydrolysis of DNA (Rao et al., 2014). Therefore this preliminary study makes an attempt to study the comparison between H and E and crystal violet in odontogenic tumors with aggressive behaviour which has not been studied so far.

**MATERIALS AND METHODS**

This retrospective case control study includes archival samples comprising of 30 cases in study group which includes benign odontogenic tumors with clinically aggressive behaviour. The control group was divided into two groups, the positive control and negative control. The positive control group comprised of clinically and histologically confirmed 10 cases of squamous cell carcinoma and the negative control group comprised of 10 cases of normal mucosa of a healthy individual. All the study samples were formalin fixed, routinely processed and embedded in paraffin. Two serial sections of thickness 5 µ were made from each specimen. One specimen was stained with H and E and another section was stained with 1 % crystal violet stain (Research Lab and chemicals.) The 1 % crystal violet stain was prepared according to the method standardised by Godkar et al (2003). The stained tissue sections were visualised in 40 X & 100 X magnifications under Leica Research Microscope. The entire tissue section was scanned. 5 areas were randomly selected randomly and mitotic figures were located with their stages of mitosis such as anaphase, prophase, metaphase & telophase and number of mitosis was recorded. All the slides were observed by 2 observers separately and the calculated data was statistically analysed. P-value of <0.05 was considered as statistically significant. All the data analysis was done using Statistical Package for Social Sciences (SPSS) (Version 15).

**OBSERVATION AND RESULTS**

When the frequency of mitotic figures were analysed in the study group and control group using both the stains, there was a significant increase in the number of mitotic figures. There was a significant statistical difference between H&E and Crystal Violet stain at 95% Confidence Interval. The control group showed presence of mitotic figures in all the phases of mitosis (Fig 1a, Fig 1b, Fig 1c and Fig 1d).

Also, abnormal mitotic figures were also visible in the control group (Fig 2). In the study group, the crystal violet showed increase in the number of mitotic figures as compared to the H and E (p<0.003).
Table 1. Distribution of mitotic figures indisease group using H & E & Crystal Violet with different phases of mitosis

<table>
<thead>
<tr>
<th>Paired Samples Test</th>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>Upper</td>
<td>Lower</td>
</tr>
<tr>
<td>Pair 1</td>
<td>-2.250</td>
<td>0.500</td>
<td>0.250</td>
<td>-3.046</td>
<td>-1.454</td>
</tr>
</tbody>
</table>

Table 2. Distribution of mitotic figures in negative control group using H & E & Crystal Violet with different phases of mitosis

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>Upper</td>
</tr>
<tr>
<td>H&amp;E Negative Control - Crystal Violet Negative Control</td>
<td>-1.500</td>
<td>0.577</td>
<td>0.289</td>
<td>-2.419</td>
</tr>
</tbody>
</table>

Table 3. Distribution of mitotic figures in positive control group using H & E & Crystal Violet with different phases of mitosis

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>Upper</td>
</tr>
<tr>
<td>H&amp;E Positive Control - Crystal Violet Positive Control</td>
<td>-4.500</td>
<td>2.380</td>
<td>1.190</td>
<td>-8.288</td>
</tr>
</tbody>
</table>

Fig 3. (a). H and E stained section of Ameloblastoma showing absence of mitotic figures. (arrow) (H and E, ×100) (b). Crystal violet stained section of Ameloblastoma showing telophase (arrow) (Crystal violet, ×100)

Fig 4: (a). H and E stained section of KCOT showing indistinct of mitotic figures. (Arrow) (H and E, ×100) (b). Crystal violet stained section of KCOT showing prophase (arrow) (Crystal violet, ×100)
The disease group shows more number of mitotic figures in anaphase and telophase per high power field recorded (Table 1) (Fig 3a, 3b, 4a & 4b). Similarly, more number of mitotic figures were seen in crystal violet stain as compared to conventional H and E, and statistically significant difference was seen both the groups i.e. in the negative control (p<0.001) (Table 2) and positive control (p<0.003) (Table 3).

DISCUSSION

Cell division is an important factor to maintain tissue integrity. In cancer abnormal cell growth and cell division results in excessive cellular proliferation. Dysplasia is associated with altered tissue architecture including cellular proliferation leading to the malignant transformation if left untreated. (Radhika, 2014) Therefore mitotic figures should be carefully evaluated to assess the cellular proliferation. (Ankle et al., 2007) Thus identification and quantitation of the mitotic figures is mandatory to study the prognosis of the precancerous and cancerous lesions in the oral activity. Various authors like Mehta et al and Mujib et al have reported the presence of mitosis in oral precancerous and cancerous lesion (Jadhav et al., 2012). We address our study to identify the mitotic figures in odontogenic tumors with aggressive behaviour. To our knowledge very few studies have been conducted to study the mitotic figures in benign and malignant odontogenic tumors. Recently, Z Sabina and Slootweg et al have conducted a study to identify mitotic figures in benign and malignant odontogenic tumors using the Hand E stain (Sabina and Slootweg, 2009).

Advanced prognostic indicators like immunohistochemistry, flow cytometry, autoradiography, and DNA ploidy are in the forefront. Since these newer techniques are costly, technique sensitive and time consuming, special stains such as crystal violet, malachite green, toluidine blue and giemsa are now being used. These stains have been applied in very few studies in the oral lesions (Kapoor et al., 2013). The aim of this study is to compare the staining of the mitotic figures of mitotic figures in odontogenic tumors with aggressive behaviour using H and E and 1% crystal violet stain. Crystal violet is a basic dye which has high affinity for the highly acidic nature of chromatin present in the mitotic cells. These mitotic cells stain magenta and stand out against the light blue background (Chieco et al., 1993).

In positive control group numerous mitotic figures were evident in all phases of cell cycle using crystal violet stain which were not clearly evident in Hand E stained section. The mitotic figures were seen in prophase, metaphase, anaphase and telophase in positive control group. Additionally we could also identify atypical mitotic figures like tripolar nuclei in the positive control group. Rarely, mitotic figures are seen in ameloblastomas. In the cases studied, Ameloblastoma showed few of mitotic figures in anaphase in crystal violet stain, which were not clearly evident in H and E stained slides (Barnes et al., 2005). KCOT have higher mitotic activity than other cysts, with a greater tendency to evolve into squamous cell carcinoma has been reported. In a study group, more number of mitotic figures in anaphase and telophase were seen in KCOT, using crystal violet stain which was difficult to identify using H and E (Gonzalez-Alva et al., 2008). In primary intraosseous carcinoma arise from residual periapical cysts, dentigerous cysts, and KCOT (OKCs), such lesions are called PIOUSC ex odontogenic cyst. Amongst all these cysts, KCOT’s seems to have higher mitotic activity than the other odontogenic cyst and has a greater potential to evolve into squamous cell carcinoma and although rare has been mentioned in literature (Tamgadge et al., 2013). In our study group, primary intraosseous carcinoma has shown mitotic figures in anaphase using crystal violet stain as compared to H and E. When compared to the other stains and advanced techniques, the crystal violet stain is economical, rapid, and reproducible. Hence, crystal violet can be used as a selective stain to study the mitotic figures.

Conclusion

Being a DNA staining material, 1% crystal violet can be used in comparison to H and E as it is economical, cheap and rapid in use. Since, we are studying only the small part of the tumour, it is difficult for us to confirm the presence of mitotic figures. Large sample size with equal number of all the lesions for study group, as well as additional comparison with the immunohistochemical markers is mandatory to decide the reliability of the crystal violet stain over the conventional H and E.

REFERENCES


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