



ISSN: 0975-833X

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

^{1,*}Tamgadge Sandhya, ²Tamgadge Avinash, ³Kashmira Kesarkar and ⁴Siddharth Acharya

¹Prof and PG Guide, Dept of Oral and Maxillofacial Pathology and Microbiology Padmashree Dr D Y Patil Dental College & Hospital, Sector 7, Nerul, Navi Mumbai, Maharashtra, India, Pin- 400706

²Prof and HOD, Dept of Oral & Maxillofacial Pathology and Microbiology, Padmashree Dr D Y Patil Dental College & Hospital, Sector 7, Nerul, Navi Mumbai, Maharashtra, India, Pin- 400706

³PG Student, Dept of Oral & Maxillofacial Pathology and Microbiology, Padmashree Dr D Y Patil Dental College & Hospital, Sector 7, Nerul, Navi Mumbai, Maharashtra, India, Pin- 400706

⁴Senior Lecturer, Dept of Public health dentistry, Padmashree Dr D Y Patil Dental College & Hospital, Sector 7, Nerul, Navi Mumbai, Maharashtra, India, Pin- 400706

ARTICLE INFO

Article History:

Received 24th March, 2016

Received in revised form

16th April, 2016

Accepted 18th May, 2016

Published online 15th June, 2016

Key words:

Cell Cycle,
Mitotic Index,
Phases of Cell Cycle,
Crystal Violet,
Aggressive Odontogenic Tumors.

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.

Copyright©2016, Tamgadge Sandhya et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Tamgadge Sandhya, Tamgadge Avinash, Kashmira kesarkar and Siddharth Acharya, 2016. "Comparison of staining of mitotic figures by h and e and crystal violet stains in odontogenic tumors with aggressive behaviour- a preliminary study", *International Journal of Current Research*, 8, (06), 32658-32662.

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).

*Corresponding author: Tamgadge Sandhya,

Prof and PG Guide, Dept of Oral and Maxillofacial Pathology and Microbiology Padmashree Dr D Y Patil Dental College & Hospital, Sector 7, Nerul, Navi Mumbai, Maharashtra, India, Pin- 400706.

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).

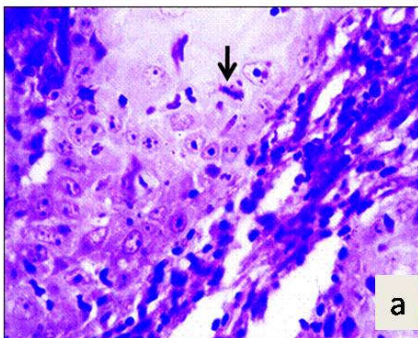


Fig 1(a). Crystal violet stained section of OSCC (positive control) showing metaphase (Crystal violet, $\times 100$)

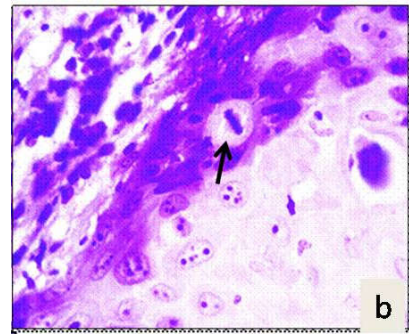


Fig 1(b) Crystal violet stained section of OSCC (positive control) showing metaphase (Crystal violet, $\times 100$)

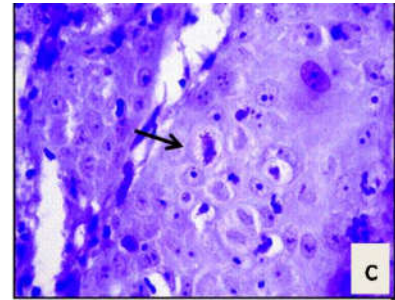


Fig 1 (c) Crystal violet stained section of OSCC (positive control) showing prophase (Crystal violet, $\times 100$)

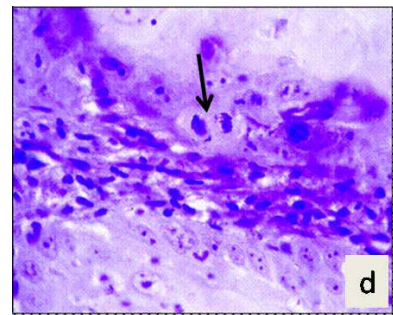


Fig 1 (d) Crystal violet stained section of OSCC (positive control) showing telophase (Crystal violet, $\times 100$)

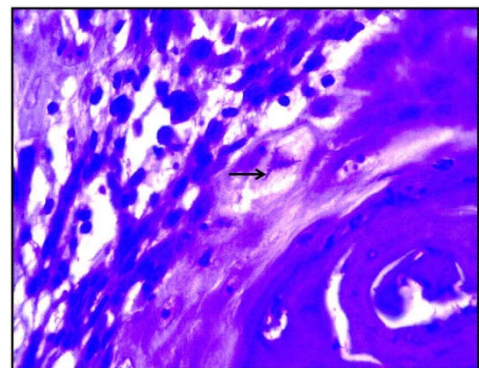


Fig 2 Crystal violet stained section of OSCC showing atypical mitotic figures that is the presence of tripolar nuclei. (Crystal violet, $\times 100$)

Also, abnormal mitotic figures were also visible in the control group (Fig 2). In the studygroup, 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

Paired Samples Test		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Upper	Lower			
Pair 1	H&E Negative Disease - Crystal Violet Disease	-2.250	0.500	0.250	-3.046	-1.454	-9.000	3	0.003

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

Paired Samples Test		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Upper	Lower			
Pair 1	H&E Negative Control - Crystal Violet Negative Control	-1.500	0.577	0.289	-2.419	-0.581	-5.196	3	0.014

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

Paired Samples Test		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Upper	Lower			
Pair 1	H&E Positive Control - Crystal Violet Positive Control	-4.500	2.380	1.190	-8.288	-0.712	-3.781	3	0.032

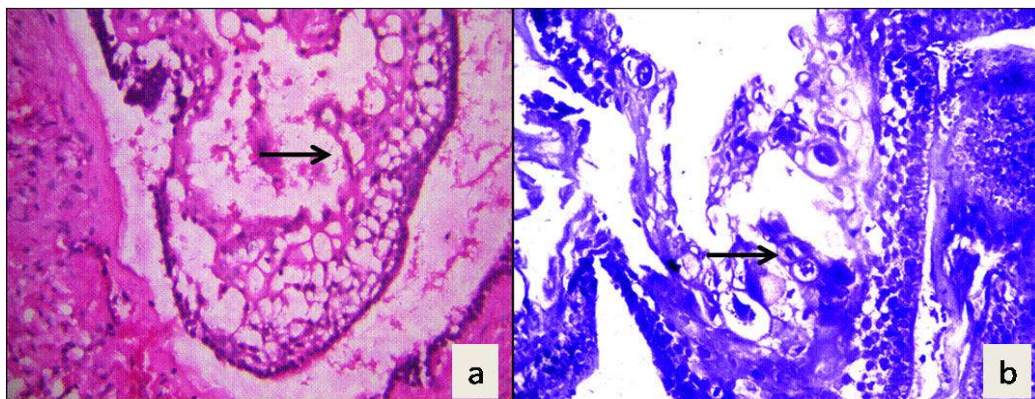


Fig 3. (a). H and E stained section of Ameloblastoma showing absence of mitotic figures. (arrow) (H and E, ×100) (b). Crystal violet stained section of Ameloblastoma showing telophase (arrow) (Crystal violet, ×100)

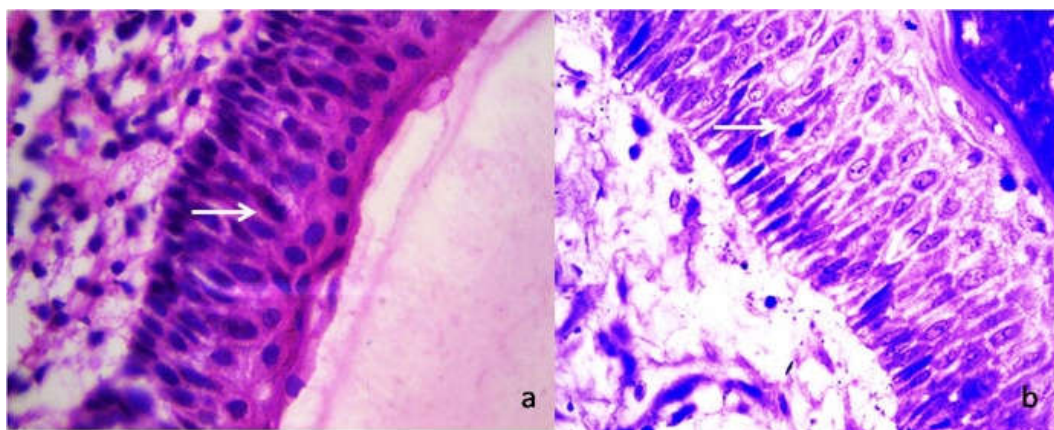


Fig 4: (a). H and E stained section of KCOT showing indistinct of mitotic figures. (Arrow) (H and E, ×100) (b). Crystal violet stained section of KCOT showing prophase (arrow) (Crystal violet, ×100)

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 frontline. 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 PIOSCC 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

- Ankle, M.R., Kale, A.D. and Charantimath, S. 2007. Comparison of staining of mitotic figures by Haematoxylin and Eosin and crystal violet stains, in oral epithelial dysplasia and squamous cell carcinoma. *Indian J Dent Res.*, 18:101-5.
- Barnes, L., Eveson, J.W., Reichart, P., Sidransky, D. 2005. (ed) Pathology and Genetics of Tumours of Head and Neck Tumours, WHO Press.
- Chieco, P., Pagnoni, M., Romagnoli, E. and Melchiorri, C. 1993. A rapid and simple Staining method, using Toluidine blue for analysing mitotic figures in tissue sections. *Histochem J.*, 25:569-77.
- Godkar, P.B. and Godkar, D.P. 2003. editors. Text book of Medical Laboratory technology. 2nd edition. Mumbai: Bhalani Publishing House; 2003.
- Gonzalez-Alva, P., Tanaka, A., Oku, Y., Yoshizawa, D., Itoh, S., Sakashita, H. *et al.* 2008. Keratocystic odontogenic tumours: a retrospective study of 183 cases. *J Oral Sci.*, 5:205-12.
- Jadhav, K.B., Ahmed Mujib, B.R. and Gupta, N. 2013. Crystal violet stain as a selective stain for the assessment of mitotic figures in oral epithelial dysplasia and oral squamous cell carcinoma. *Indian J Pathol Microbiol*, 55:283-7.
- Kapoor, K., Puri, A., Prakash, A., Jazib, Sharma G. 2013. Mitotic Counting and its significance in Histopathological Grading of OSCC & Oral Epithelial Dysplasia. *Heal Talk* 05:35-37.
- Palaskar, S., Patil, S., Narang, B., Prabhu, P., Kathuriya P. and Pawar, R. 2013. Efficacy of various stains to study mitotic figures in oral epithelial dysplasia –A pilot study. *J Dent Allied Sci.*, 2:46-48

- Radhika, B.M. 2014. Mitosis at a glance. *J Oral Maxillofac Pathol*, 18: S2-S5.
- Rao, R.S., Patil, S. and Agarwal, A. 2014. Comparison and evaluation of mitotic figures in oral epithelial dysplasia using crystal vital and Feulgen stain. *The Journal of Contemporary Dental Practice*, 15:273-274.
- Sabina, Z. and Sloodweg, P. J. 2009. Mitotic index in clear cell odontogenic tumors. *Therapeutics, Pharmacology and Clinical Toxicology*, 13: 172-178.
- Tamgadge, S., Tamgadge, A., Modak, N. and Bhalerao, S. 2013. Primary intraosseous squamous cell carcinoma arising from an odontogenic keratocyst: a case report and literature review. *ecancermedicalscience*. 2013; 7:316. doi:10.3332/ecancer.2013.316.
