Int.J.Curr.Microbiol.App.Sci (2017) *6*(3): 687-693

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 3 (2017) pp. 687-693**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.603.079

Comparison of Staining of Keratin Pearl and Individual Cell Keratin in Oral Squamous Cell Carcinoma by Modified Papanicolaou, Modified Mallory's and Hematoxylin and Eosin Stain

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Keywords

Squamous cell carcinoma (SCC), Keratin Pearl (KP), Individual cell Keratin (ICK), Modified Papanicolou (PAP) stain, Hematoxylin and Eosin (H&E) stain.

Article Info

Accepted:
15 February 2017
Available Online:
10 March 2017

A B S T R A C T

Observation of Keratin pearl (KP) and individual cell keratin (ICK) is one of the criteria in grading oral squamous cell carcinoma (SCC). The aim of the study was to evaluate and compare the distinct staining and identification of KP and ICK by routine Hematoxylin and Eosin (H&E) stain Modified Mallory's and Modified Papanicolaou Stain. A total number of 38 paraffin embedded tissues of known cases of well-differentiated and moderately-differentiated SCC were taken and 3 sections of 4-5 micron thickness from each block were cut and stained with H&E stain, Modified Mallory's and modified Papanicolou stain. The KP and ICK were distinctly and clearly stained by modified Papanicolou, modified Mallory's stain compared to H&E stain. The positive staining of KP and ICK by modified Papanicolou stain was statistically significant than modified Mallory's and H&E stain at P=0.033 and P=0.001. Based on our findings we conclude that the efficacy of distinct identification of KP and ICK in oral SCC by modified Papanicolou stain is better than modified Mallory's stain and H&E stain. So it can be used as an adjuvant in case of oral SCC.

Introduction

Oral cancer is one of the most common malignant tumours. Oral squamous cell carcinoma constitutes more than 90% of the oral cancer. It is mostly reported at late stage in Indian scenario (Neville *et al*., 2002; Shafer *et al*., 1993; Parija, 1991; Pindborg *et al*., 1990). The prognosis and treatment outcome is proportional to differentiation level of OSCC. A well differentiated OSCC has better prognosis (Harrison *et al*., 1999; Kumar *et al*.,

2004). One of the important parameter in grading is keratin pearl and individual cell keratin observation (Neville *et al*., 2009). The quality and quantity of keratin synthesis reflects the differentiation level of normal and abnormal epithelial cells (Coulombe *et al*., 1990; Schweizer *et al*., 1983; Nagle *et al*., 1983; Rothman *et al*., 1954).

The identification and staining of individual cell keratin and keratin pearl is important in histopathological grading and diagnosis of oral squamous cell carcinoma (Neville *et al*., 2002; Shafer *et al*., 1993; Parija, 1991; Pindborg *et al*., 1990).

So we studied and compared the identification and staining of individual cell keratin and keratin pearl in oral squamous cell carcinoma by routine Hematoxylin & Eosin stain modified Papanicolaou stain and modified Mallory's stain (Ayoub *et al*., 1963).

Materials and Methods

A total numbers of 38 cases of OSCC were taken for study. The histo-pathologically diagnosed cases of oral squamous cell carcinomas from were retrieved from pathology department for the study group. The only criteria selected for inclusion is that there should be enough tissue material in paraffin blocks. From each block three serial sections were made of 5-micron thickness and stained by routine H&E stain, modified Mallory's stain and modified Papanicolou stain for keratin.

The staining protocol suggested by Richard P. Elzay (Elzay *et al*., 1983) for modified Papanicolou stain and Ayoub-shklar for modified Mallory's stain was followed for all the cases.

The criteria of clear identification of keratin pearl and individual cell keratin were analyzed with an aim to identify and compare the better staining of keratin by H&E stain and modified Papanicolou stain and modified Mallory's stain in oral squamous cell carcinoma.

The data was subjected to statistical analysis by SPSS software version 16.

Results and Discussion

The study included 38 cases of oral SCC which were stained by H&E, modified mallory's and modified Papanicolou stain. These stained sections were evaluated and compared for distinct and clear identification of KP and ICK.

The modified papanicolou stain showed a positive staining of KP in 30 (78.9%) cases, modified Mallory's stain showed positivity in 28(73.7%) cases H&E stain showed positive staining in 20(52.6%) cases (Table-1, Graph-1).

The positive staining of KP was statistically significant for all stains with chi-square value of 6.821, p value of $p < 0.05$ ($p=0.033$) (Table-3). This Indicates there was a statistically significant positive staining of KP by modified Papanicolou stain than modified Mallory and H&E stain in oral SCC.

The modified PAP stain showed a positive staining of ICK in 30 (78.9%) cases and Modified Mallory's stain showed positivety in 23 (60.53%) cases whereas H&E stain showed positive staining in 13 (34.2%) cases (Table-2, Graph-2).

The positive staining of ICK was statistically significant for modified PAP than H&E with chi-square value of 15.761, p value of $p = 0.001$ (Table-4). This Indicates there was a statistically significant positive staining of ICK by modified Papanicolou than modified Mallory's and H&E stain in oral SCC.

In squamous cell carcinoma level of keratin synthesis is directly proportional to the level of differentiation which has an association with prognosis. Hence the study was taken up with an aim to compare clear and better identification keratin pearl and individual cell keratinization in the oral squamous cell carcinoma with H&E and modified Papanicolou and modified Mallory's stain. The demonstration of keratin pearl was significantly positive at $p < 0.05$ in Modified Papanicolaou stain than modified Mallory's stain and H&E stain. This can be utilized for the easy confirmation of diagnosis a well-differentiated SCC.

Table.1 Keratin Pearl staining in oral squamous cell carcinoma by hematoxylin and eosin stain, modified Papanicolaou stain and modified Mallory's stain

	H&E Stain	Modified Papanicolou Stain	Modified Mallory's Stain
Positive Staininig	20	30	28
Negative Staining	18	8	10
TOTAL	38	38	38

Table.2 Individual cell Keratin staining in oral squamous cell carcinoma by hematoxylin and eosin stain, modified Papanicolaou stain and modified Mallory's stain

	H&E Stain	Modified Papanicolou Stain	Modified Mallory's Stain
Positive Staininig	13	30	23
Negative Staining	25	8	15
TOTAL	38	38	38

Table.3 Statistical analysis of Keratin Pearl staining in oral squamous cell carcinoma by hematoxylin and eosin stain, modified Papanicolaou stain and modified Mallory's stain

Count		H & E Stain	Modified papanicolou stain	Modified Mallory Stain
	Positive	20	30	28
	Negative	18	8	10
Total		38	38	38

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.821[a]	2	.033
Likelihood Ratio	6.704	2	.035
Linear-by-Linear Association	3.863	1	.049
N of Valid Cases	114		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 12.00.

Table.4 Individual cell Keratin staining in oral squamous cell carcinoma by hematoxylin and eosin stain, modified Papanicolaou stain and modified Mallory's stain

Count	H & E Stain	Modified papanicolou stain	Modified Mallory Stain
Positive	13	30	23
Negative	25	8	15
Total	38	38	38

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.761[a]	2	.001
Likelihood Ratio	16.264	2	.000
Linear-by-Linear Association	5.350	1	.021
N of Valid Cases	114		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.00.

Fig.1 Keratin pearl staining in oral squamous cell carcinoma by H&E (a) and Modified Papanicolaou (b) and Modified Mallory's (c) stain

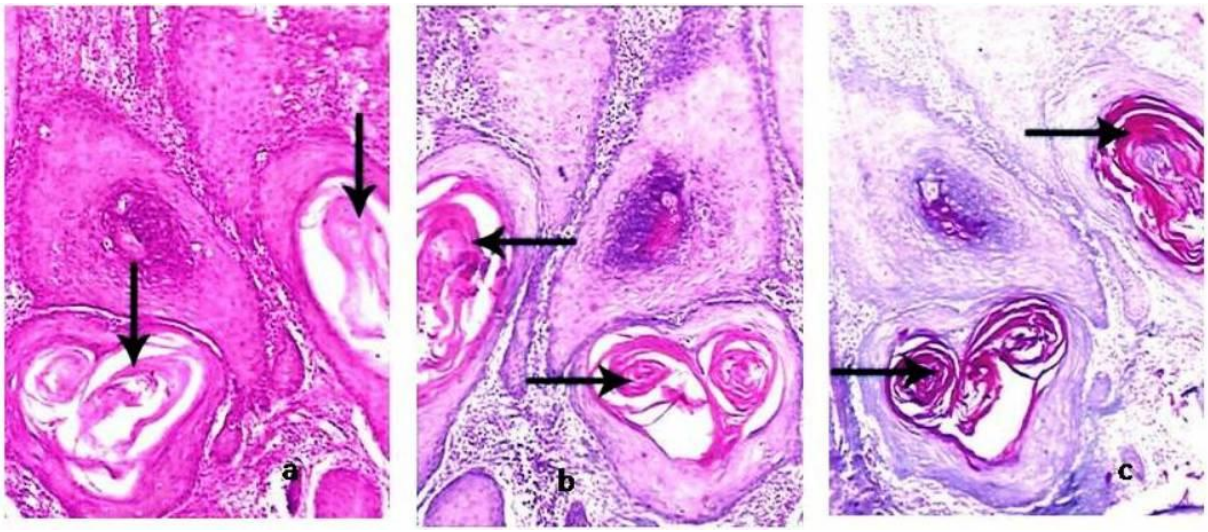
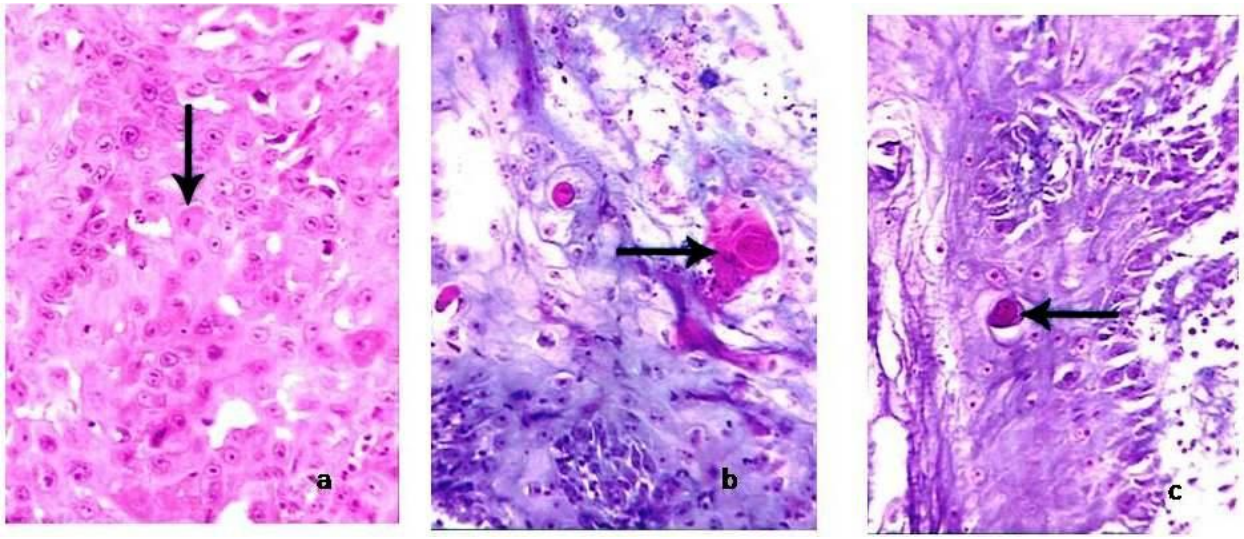
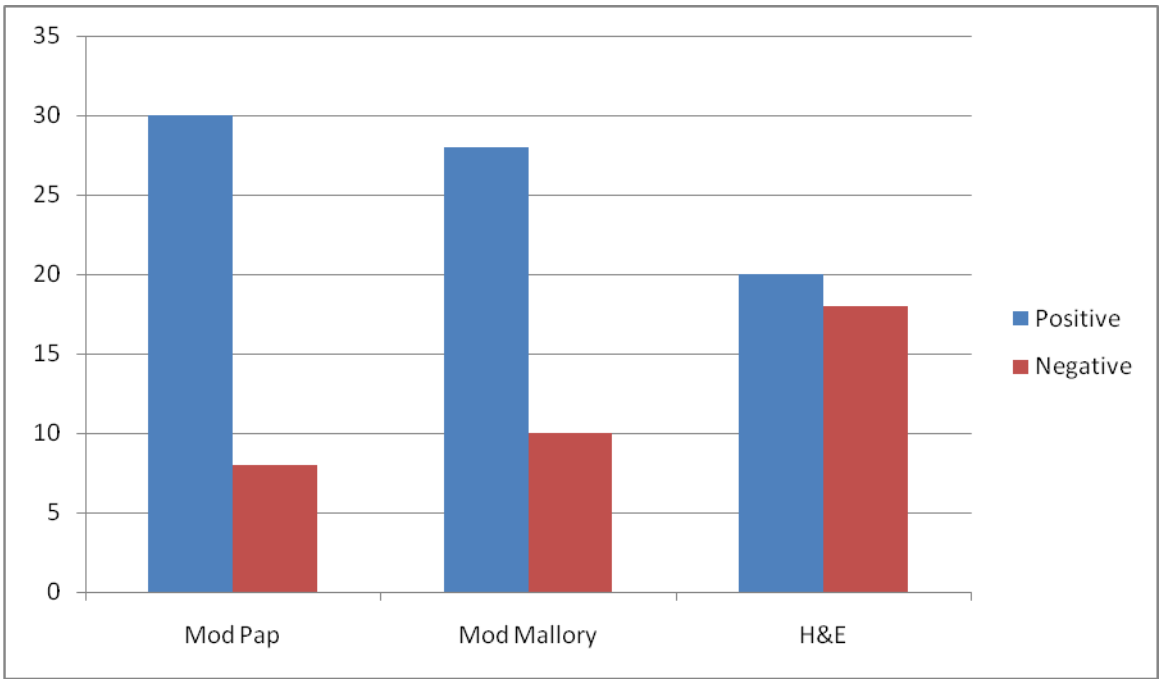


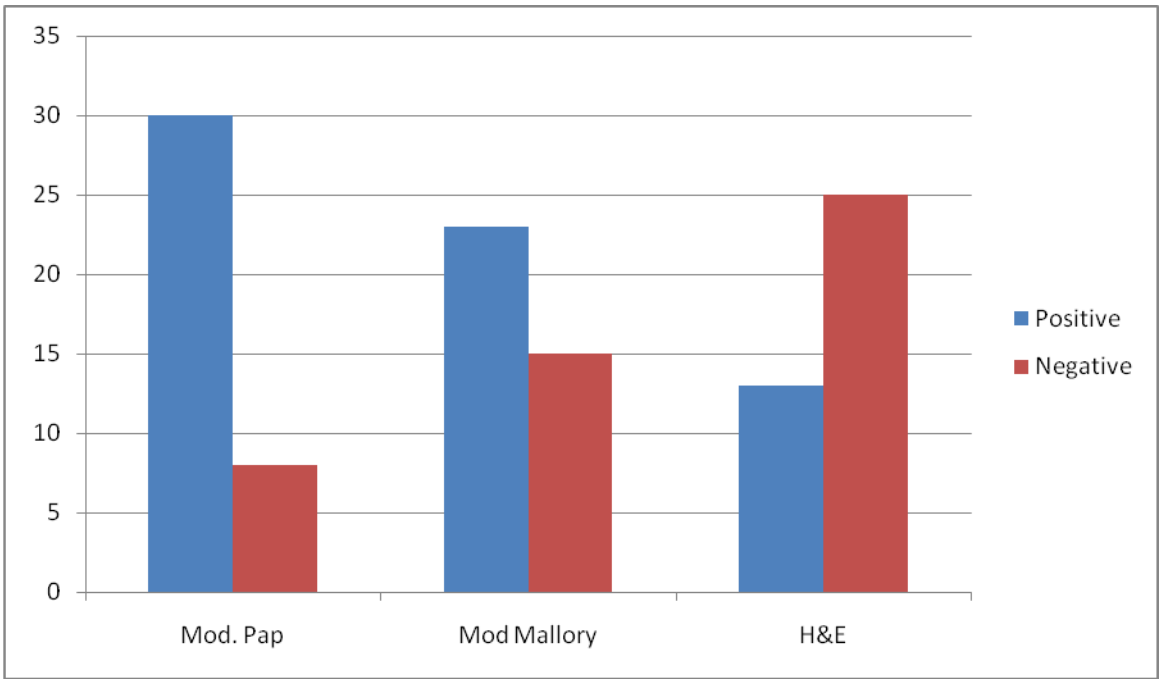
Fig.2 Individual Cell Keratin staining in oral squamous cell carcinoma by H&E (a) Modified Papanicolaou (b) and Modified Mallory's (c) stain



Graph.1 Keratin Pearl staining by hematoxylin and eosin stain and modified Papanicolaou stain and modified Mallory stain in Normal keratinized Epithelium



Graph.2 Individual cell Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain and modified Mallory stain in Normal keratinized Epithelium



Similar finding of high degree of intensity of staining of keratin in modified Papanicolou stain was reported by Santhosh kumar *et al.*, (2016), Richard Elzay (1983) and Modified Mallory stain was reported by Ayoub-shklar. This concurs with our study.

The individual cell keratinisation was significantly positive at p = 0.001 in Modified Papanicolaou stain compared to modified Mallory's stain and H &E stain in oral SCC group. Similar findings were reported in various other studies. This value can be important in identifying differentiation level of squamous cell carcinoma.

There is varying and abnormal level of keratin expression in malignant cells of oral SCC. This different nature of keratin molecule prevailing within the dysplastic cell probably explain the difference in staining quality of modified Papanicolaou stain mainly as well as modified Mallory's stain as shown in our study.

This variation in staining quality depends on degree of variation of keratinisation during the progression of the malignancy.

The Modified Papanicolaou stain has more significant association with demonstration of keratin pearl and individual cell keratinization in well and moderately differentiated squamous cell carcinoma than the routine H & E stain. It can be favorably utilized to visualize the keratin pearl and individual cell keratin areas, by doing so the differentiation level of epithelial cells in oral squamous cell carcinoma.

We conclude that the efficacy of distinct identification of KP and ICK in oral SCC by modified PAP stain is better than H&E stain. But H&E is gold standard and simple stain in demonstrating other details like nucleus, connective tissue structures. So modified PAP can be used as an adjuvant stain in case of oral SCC along with H&E stain.

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How to cite this article:

Santhosh Kumar Caliaperoumal, Sudhakar Srinivasan, Meer Ahmed Ibrahim Munshi. 2017. Comparison of Staining of Keratin Pearl and Individual Cell Keratin in Oral Squamous Cell Carcinoma by Modified Papanicolaou, Modified Mallory's and Hematoxylin and Eosin Stain. *Int.J.Curr.Microbiol.App.Sci.* 6(3): 687-693. doi: https://doi.org/10.20546/ijcmas.2017.603.079