

Original Research Article

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## Comparison of Modified Papanicolaou and Hematoxylin and Eosin Stain in Demonstration of Keratin Pearl and Individual Cell Keratin in Oral Squamous Cell Carcinoma

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### ABSTRACT

#### Keywords

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

#### Article Info

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Keratin pearl (KP) and individual cell keratin(ICK) observation 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 & Eosin (H&E) stain and Modified Papanicolaou (PAP) Stain. A total number of 38 paraffin embedded tissues of known cases of well-differentiated and moderately-differentiated SCC were taken and 2 sections of 4-5 micron thickness from each block were cut and stained with H&E stain and modified PAP stain. The KP and ICK were distinctly and clearly stained by modified PAP compared to H&E stain. The positive staining of KP and ICK by modified PAP was statistically significant with  $P=0.001$ ,  $f=0.001$  and  $P=0.022$ ,  $f=0.034$  than H&E stain. Based on our findings 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. So it can be used as an adjuvant in case of oral SCC.

### Introduction

Keratin is an intermediate filamentous protein generally found in the surface epithelium (Clausen *et al.*, 1986; Steinert *et al.*, 1995; Coulombe *et al.*, 1990; Neville *et al.*, 2002). Their role is to protect the underlying structures, which is one of the

prime functions of surface epithelium (Tencate, 1998). The keratin synthesis reflects the differentiation level of normal epithelial cell as well as the malignant epithelial cells of carcinoma (Schweizer *et al.*, 1983; Gould *et al.*, 1985; Nagle *et al.*, 1983 ; Pindborg *et al.*, 1990).

It is generally understood that the more differentiation a cell exhibit in the case of neoplasm, better is the prognosis. Hence a thorough knowledge is needed to analyse the differentiation pattern of a neoplasm, which will help in the planning the treatment of particular neoplasm (Harrison *et al.*, 1999; Neville *et al.*, 2002; Neville *et al.*, 2002; v *et al.*, 1954; Santis *et al.*, 1964).

Oral squamous cell carcinoma is one of the commonest tumor which is usually reported at very advanced stage (Harrison, 1999; Neville *et al.*, 2002; Shafer *et al.*, 1993; Rothman, 1954). If we could understand the differentiation level of the tumor, we can plan the treatment modalities in a better prospect for which the level of keratin synthesis by malignant epithelial cell would be a good predictor.

Hence, it is proposed to take up the study of keratin pearl and individual cell keratin in oral squamous cell carcinoma by utilizing modified Papanicolaou stain and to compare it with H & E stain (Elzay, 1983; Drijver *et al.*, 1983; Culling, 1963; Bancroft *et al.*, 2002; Papanicolaou, 1941, 1942).

The aim of this study was to evaluate and compare the distinct identification of keratin pearl and individual cell keratin using the hematoxylin and eosin stain, modified Papanicolaou stain.

### **Materials and Methods**

The total number of 38 cases of carcinomas of the study group included 21 well-differentiated and 17 moderately-differentiated squamous cell carcinoma.

The histo-pathologically diagnosed cases of oral squamous cell carcinomas from “Department of Oral Pathology And Microbiology, Mahatma Gandhi Post-

Graduate Institute of Dental Sciences” were retrieved for the study group. The only criteria selected for inclusion is that there should be enough tissue material in paraffin blocks. From each block two serial sections were made of 5-micron thickness and stained by routine H&E stain and modified PAP stain for keratin.

The staining protocol suggested by Richard P. Elzay for modified Papanicolaou stain was followed for all the cases.

The following criteria were analyzed with an aim to identify and compare the better staining of keratin by H&E stain and modified Papanicolaou stain in oral squamous cell carcinoma.

### **Criteria**

A clear identification of keratin pearl and also A clear identification of Individual cell keratinisation.

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 and modified PAP stain. These stained sections were evaluated and compared for distinct and clear identification of KP and ICK.

The modified PAP stain showed a positive staining of KP in 30 (78.9%) cases, whereas H&E stain showed positive staining in 20(52.6%) cases (Table-1, Graph-1).

The positive staining of KP was statistically significant for modified PAP than H&E with chi-square value of 11.259, p value of  $p = 0.001$  and Fisher’s exact test:  $f = 0.001$  (Table-2). This Indicates there was a

statistically significant positive staining of KP by modified PAP than H&E stain in oral SCC.

The modified PAP stain showed a positive staining of ICK in 30 (78.9%) cases, whereas H&E stain showed positive staining in 13 (34.2%) cases (Table-3, Graph-2).

The positive staining of ICK was statistically significant for modified PAP than H&E with chi-square value of 5.269, p value of  $p = 0.022$  and Fisher's exact test:  $f = 0.034$  (Table-4). This Indicates there was a statistically significant positive staining of ICK by modified PAP than H&E stain in oral SCC.

In squamous cell carcinoma level of keratin synthesis reflect the level of differentiation which has an association with prognosis. Hence the study was taken up with an aim of clear and better identification keratin pearl and individual cell keratinization in the oral squamous cell carcinoma with H&E and modified Papanicolaou stain.

The control group consisting of 10 cases showed in the statistical analysis, that there is no degree of significance using Modified Papanicolaou stain in demonstrating keratin. This may probability due to paucity of number of samples taken for the control group.

The demonstration of keratin pearl was significantly positive at  $p = 0.001$  and fisher's test of  $f = 0.001$  in Modified

Papanicolaou stain compared to the H &E in oral SCC group. This can be utilized for the easy and confirm diagnosis of carcinoma at a well-differentiated stage. Similar finding of high degree of intensity of staining of keratin in modified PAP stain was reported by Richard P Elzay (1983). This concurs with our study.

The individual cell keratinisation was significantly positive at  $p < 0.05$  ( $p = 0.022$ ) and fisher's test of  $f = 0.034$  in Modified Papanicolaou stain compared to the H &E stain in oral SCC group. This value can be important in identifying differentiation level of squamous cell carcinoma.

It has been reported by Nagle *et al.*, (1996) that there was varying expression of keratin molecule existing within the malignant epithelial cells that are not present in normal epithelial cell.

This different nature of keratin molecule prevailing within the cell, probably explain the difference in staining quality of modified Papanicolaou stain as shown in our study. It was also quite interesting to note that Modified Papanicolaou stain was able to pick – up stain of those low molecular weight keratin filaments generally noticed in carcinoma cases. This variation in staining quality depends on degree of variation of keratinisation during the progression of the malignancy.

**Table.1** Keratin Pearl staining by hematoxylin and eosin stain and modified Papanicolaou stain in oral squamous cell carcinoma

	H & E Stain	Modified Papanicolou Stain
Positive Staininig	20	30
Negative Staining	18	8
TOTAL	38	38

**Table.2** Statistical analysis of Keratin Pearl Staining with H&E and Modified Papanicolou stain

<b>Cross tabulation</b>					
COUNT	Keratin Pearl Staining Mod.Pap				
Keratin Pearl Staining H&E		Negative	Positive	Total	
	Negative	8	10	18	
	Positive	0	20	20	
	Toatl	8	30	38	
<b>Chi-Square Tests</b>					
	value	df	Asymp. Sig (2-sided)	Exact Sig (2-sided)	Exact Sig (1-sided)
Pearson chi-Square	11.259	1	.001		
Fisher's Exact Test				.001	.001

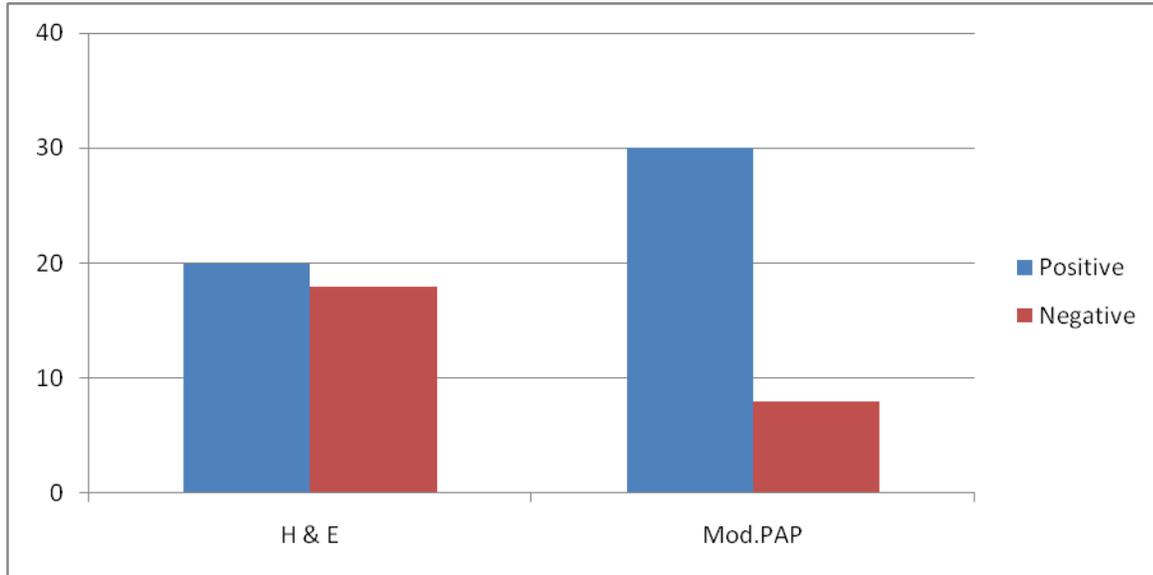
**Table.3** Individual cell Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain in oral squamous cell carcinoma

	H&E Stain	Modified Papanicolou Stain
Positive Staininig	13	30
Negative Staining	25	8
TOTAL	38	38

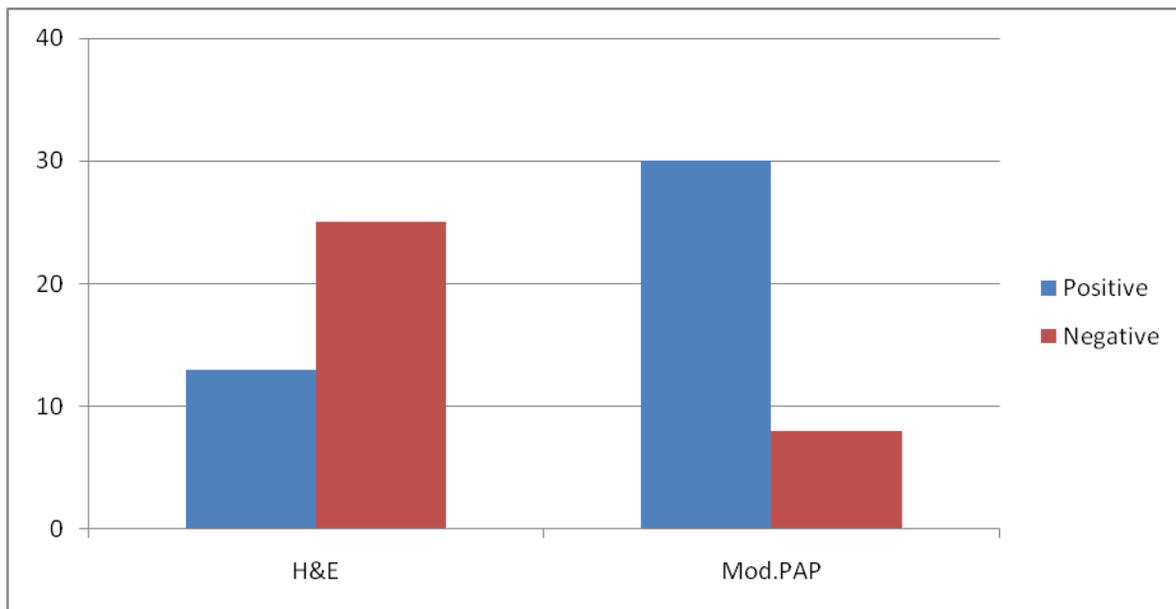
**Table.4** Statistical analysis of Individual cell Keratin Staining with H&E and Modified Papanicolou stain

<b>Cross tabulation</b>					
COUNT	Individual Cell Keratin Staining Mod.Pap				
Individual Cell Keratin Pearl Staining H&E		Negative	Positive	Total	
	Negative	8	17	25	
	Positive	0	13	13	
	Toatl	8	30	38	
<b>Chi-Square Tests</b>					
	value	df	Asymp. Sig(2-sided)	Exact Sig(2-sided)	Exact Sig(1-sided)
Pearson chi-Square	5.269	1	.022		
Fisher's Exact Test				.034	.022

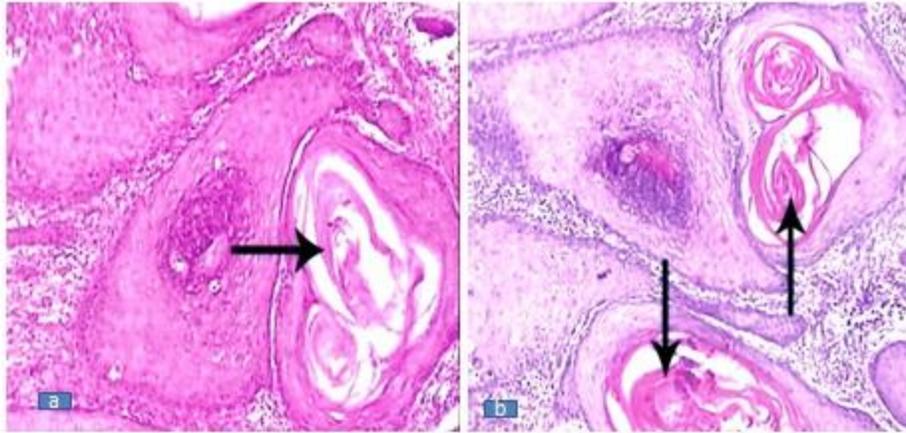
**Graph.1** Keratin Pearl staining by hematoxylin and eosin stain and modified Papanicolaou stain in oral squamous cell carcinoma



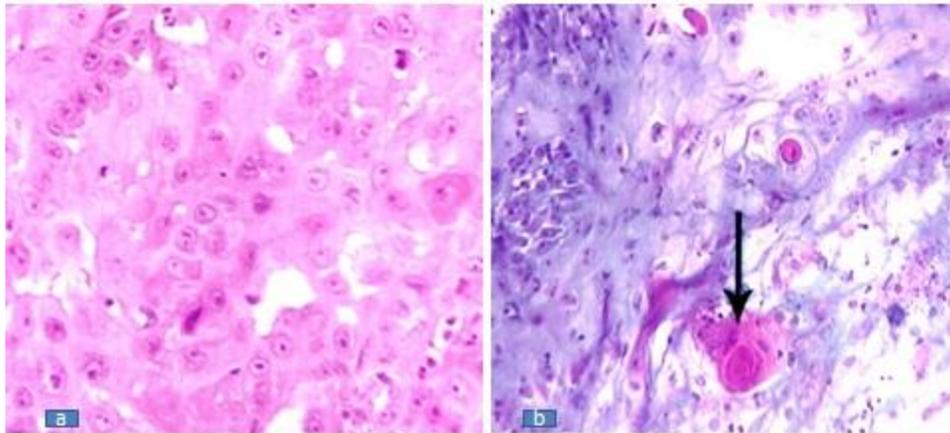
**Graph.2** Individual cell Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain in oral squamous cell carcinoma



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



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



This study has proved that the Modified Papanicolaou stain has more significant association with demonstration of keratin pearl and individual cell keratinization 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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