

Original Research Article

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## Comparison and Demonstration of Surface Keratin Staining Quality by Modified Papanicalou Stain, Modified Mallory's Stain and Hematoxylin and Eosin Stain in Verrucous Carcinoma and Normal Keratinizing Epithelium

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### ABSTRACT

#### Keywords

Verrucous carcinoma (VC), Modified Papanicolou (MP) stain, Modified Mallory's (MM) stain, Hematoxylin and Eosin (H&E) stain.

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Observation of normal or abnormal keratin forms is the major criteria in histopathological diagnosis of Verrucous carcinoma. The aim of the study is to evaluate and compare the distinct staining and surface keratin in verrucous carcinoma and normal keratinizing epithelium by routine hematoxylin and eosin (H&E) stain, modified papanicolou stain and modified Mallory's stain. The known cases of verrucous carcinoma and tissues from normal keratinizing epithelial regions with sufficient paraffin embedded tissue were selected. The three sections from each block were stained with H&E stain, modified Papanicolou stain and modified Mallory's stain. The modified Mallory's stain, modified Papanicolou stain distinctively and clearly stained surface keratin compared to H&E stain. The positive staining of surface keratin was significantly associated with all stains at the level of  $p=0.001$  in verrucous carcinoma and normal epithelium respectively. Based on our observation we conclude that the positive staining of surface keratin was significantly associated at  $p=0.001$  in verrucous carcinoma and normal epithelium with all stains however the positivity was more associated with modified Papanicolou stain followed by modified Mallory stain least by hematoxylin and eosin Stain. So, these special stains can be used as an adjuvant stain along with H&E stain.

### Introduction

Verrucous carcinoma is a unique type of early neoplasm with good prognosis and abundant keratin production on the surface (Neville *et al.*, 2009; Shafer *et al.*, 1993). This keratin plug is well demarcated and can be studied easily. Cytokeratin is an intermediate filamentous protein which matures to form keratinous superficial layer of keratinized

epithelium (Clausen *et al.*, 1986; Tencate, 1998; Steinert *et al.*, 1995; Coulombe *et al.*, 1990). The major role of keratin is to protect underlying tissue from any external physical or chemical damage (Tencate, 1998; Steinert *et al.*, 1995). The differentiation level of normal or abnormal epithelium can be assessed by the amount and quality of keratin

production (Tencate, 1998; Steinert *et al.*, 1995; Schweizer *et al.*, 1983; Gould, 1985).

The identification of keratin in routine hematoxylin and eosin stain forms a major criterion in histopathological diagnosis of oral premalignant and malignant lesions (Neville *et al.*, 2002; Harrison, 1999). Hence the special stain for keratin will help in assessing the keratin level in these lesions (Elzay, 1983; Santhosh kumaret *al.*, 2016).

So a study of keratin was done in verrucous carcinoma and normal orthokeratinized epithelium by utilizing modified Mallory connective tissue stain and modified Papanicolaou stain to assess the intensity and level of keratin staining.

The aim of this study was to evaluate and compare the distinct staining of keratin using the hematoxylin and eosin stain, modified Papanicolaou stain and modified Mallory stain.

## **Materials and Methods**

The total number of 15 cases of verrucous carcinomas of study group and 10 number of control groups of normal keratinizing tissues like gingiva, palate and skin were taken.

The histopathologically diagnosed cases of verrucous carcinomas and normal keratinizing tissues like gingiva, palate and skin were retrieved for the study. 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 and modified Papanicolaou stain and modified Mallory's stain for keratin. The staining protocol suggested by Elzay (1983) for modified Papanicolaou stain and Ayoub-shklar (1964) for modified Mallory's stain was followed for all the cases.

The criteria of clear and distinct level of staining of surface keratin through grade-1 (poor), grade-2 (moderate) and grade-3 (good) staining was analyzed with an aim to identify and compare the better staining of keratin by H&E stain, modified Papanicolaou and modified Mallory's stain in verrucous carcinoma.

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

## **Results and Discussion**

The study included 15 cases of oral VC and 10 normal orthokeratinized mucosa which were stained by H&E, modified Papanicolaou and modified Mallory's stain. These stained sections were evaluated and compared for distinct and clear identification of surface keratin.

The MP, MM and H&E stain showed a positive staining of surface Keratin in all 15 cases of VC with varying degree of positivity (Table 1, Graph 1).

The positive staining of surface keratin was statistically significant for all three stains with chi-square value of 50.090, p value of  $p = 0.001$  (Table 2). This Indicates there was a statistically significant positive staining of surface keratin by all three stains in VC.

The MP and MP stain showed a positive staining of surface Keratin in all 10 cases and H&E stain showed a positive staining of surface Keratin in 7 cases of normal epithelium with varying degree of positivity (Table 3, Graph 2).

The positive staining of surface keratin was statistically significant for all three stains with chi-square value of 32.967, p value of  $p = 0.001$  (Table 4). This Indicates there was a statistically significant positive staining of surface keratin by all three stains in normal

epithelium. Verrucous carcinoma is an early form of oral carcinoma with good prognosis. These lesions shows abundant abnormal surface keratin layer and keratin plugs. In any normal or abnormal epithelium the level of keratin synthesis reflect the level of differentiation which has an association with prognosis.

The study was done to analyse and compare the better staining and identification of surface keratin with MP, MM and H&E stain in Verrucous carcinoma and normal keratinizing tissues like gingiva, palate and skin.

The MP, MM and H&E stain showed a positive staining of surface Keratin in all 15 cases of VC with varying degree of positivity (Table 1, Graph 1).

The positive staining of surface keratin was statistically significant for all three stains with chi-square value of 50.090, p value of  $p = 0.001$  (Table 2). This Indicates there was a statistically significant positive staining of surface keratin by all three stains in VC.

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epithelium with varying degree of positivity (Table 3, Graph 2).

The positive staining of surface keratin was statistically significant for all three stains with chi-square value of 32.967, p value of  $p = 0.001$  (Table 4). This Indicates there was a statistically significant positive staining of surface keratin by all three stains in normal epithelium.

These stains can be utilized for the easy and confirm the presence of keratin in diagnosis of carcinoma at a well-differentiated stage. Similar finding of high degree of intensity of staining of keratin was reported in many other studies.

It has been reported 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 and 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.

**Table.1** Surface Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain and modified Mallory stain in verrucous carcinoma

		H&E Stain	Modified Papanicolou Stain	Modified Mallory stain
Positive Grade of Staining	Grade-1	15	0	0
	Grade-2	0	9	2
	Grade-3	0	6	13
Negative staining		0	0	0
TOTAL		15	15	15

**Table.2** Statistical analysis (with SPSS16) of Surface Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain and modified Mallory stain in verrucous carcinoma

		H & E Stain	Modified Papanicolaou stain	Modified Mallory Stain
Positive	Grade I	15	0	0
	Grade II	0	9	4
	Grade III	0	6	11
Total		15	15	15

**Chi-Square Tests**

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	50.090 <sup>a</sup>	4	.001
Likelihood Ratio	60.752	4	.000
Linear-by-Linear Association	31.070	1	.000
N of Valid Cases	45		

a. 3 cells (33.3%) have expected count less than 5. The minimum expected count is 4.33.

**Table.3** Surface Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain and modified Mallory stain in Normal keratinized Epithelium

		H&E Stain	Modified Papanicolou Stain	Modified Mallory stain
Positive Grade of Staininig	Grade-1	7	0	0
	Grade-2	0	8	5
	Grade-3	0	2	5
Negative staining		3	0	0
TOTAL		10	10	10

**Table.4** Statistical analysis (with SPSS16) of Surface Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain and modified Mallory stain in Normal Epithelium

		H & E Stain	Modified Papanicolaou stain	Modified Mallory Stain
Positive	Grade I	7	0	0
	Grade II	0	8	5
	Grade III	0	2	5
	Negative	3	0	0

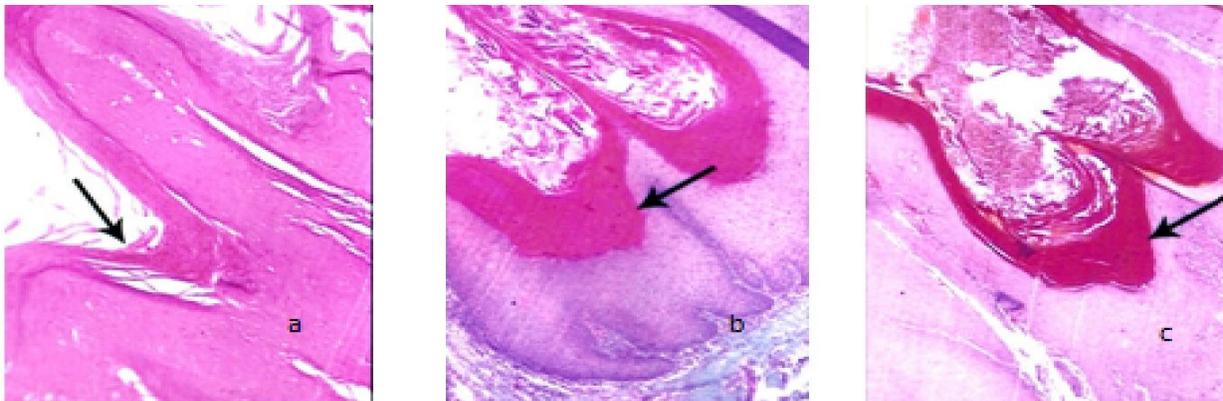
		H & E Stain	Modified Papanicolaou stain	Modified Mallory Stain
Positive	Grade I	7	0	0
	Grade II	0	8	5
	Grade III	0	2	5
	Negative	3	0	0
Total		10	10	10

**Chi-Square Tests**

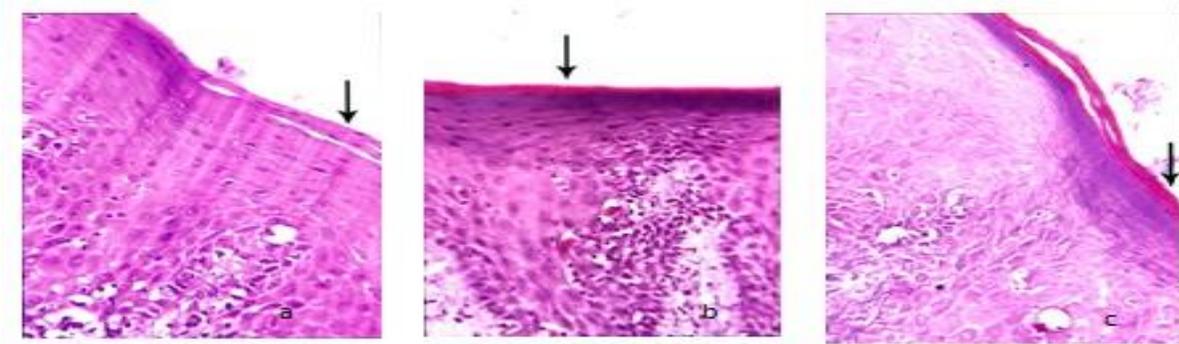
	Value	df	Asymp. Sig. (2-sided)
<b>Pearson Chi-Square</b>	<b>32.967<sup>a</sup></b>	<b>6</b>	<b>.001</b>
Likelihood Ratio	40.218	6	.000
Linear-by-Linear Association	2.105	1	.147
N of Valid Cases	30		

a. 12 cells (100.0%) have expected count less than 5. The minimum expected count is 1.00.

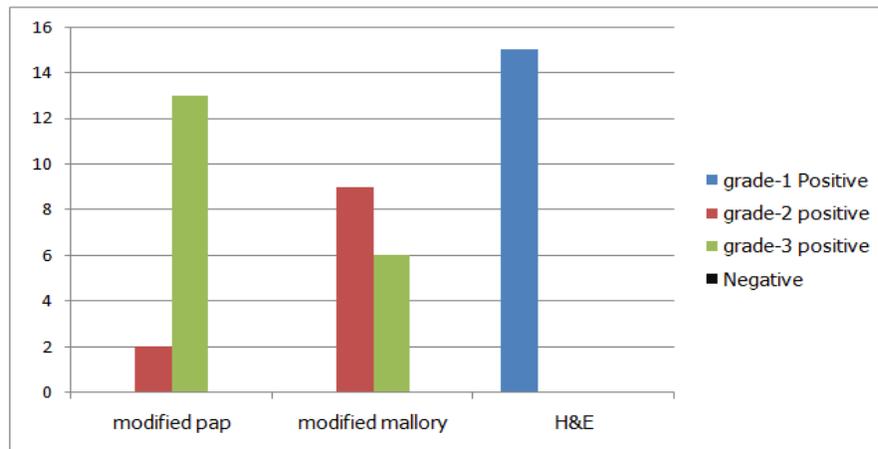
**Figure.1** Surface Keratin staining in verrucous carcinoma by H&E (a), Modified Papanicolaou (b) and modified Mallory's (c) stain



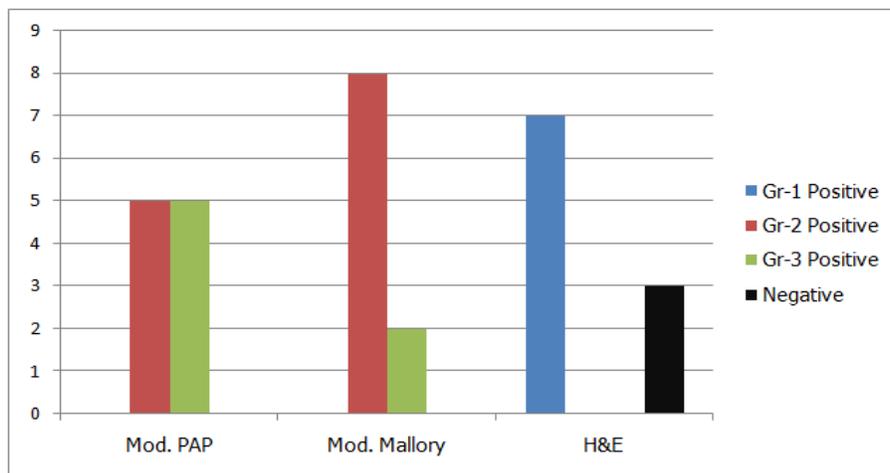
**Figure.2** Surface Keratin staining in Keratinized epithelium by H&E (a), Modified Papanicolaou (b) and modified Mallory's (c) stain



**Graph.1** Surface Keratin staining by hematoxylin and eosin stain and modified Papanicolaou stain and modified Mallory stain in verrucous carcinoma



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



This study has proved that the Modified Papanicolaou stain and modified Mallory's stain has more significant staining of surface keratin than the routine H & E stain. The present study concluded that the efficacy of distinct staining and identification of surface keratin in verrucous carcinoma and normal keratinized epithelium by MP, MM and H&E stain is statistically significant. So MP and MM can be used as an adjuvant stain in case where keratin demonstration has importance in diagnosis and prognosis along with H&E stain. But H&E stain is gold standard and simple stain in demonstrating other details like nucleus, connective tissue structures.

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