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# Staining Effectiveness of Keratin Using Hematoxylin & Eosin and Special Stains: A Comparative Study

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## Abstract

**Introduction:** Keratin is a stiff fibrous structural protein, which is present in epithelial cells and creates intermediate filaments that give tissues strength, rigidity and protection. Because of its high cysteine content, keratin can form strong disulfide connections that make it extremely resilient and long-lasting. Defects in the keratinization process cause a variety of inherited and premalignant diseases hence keratin can be used as crucial diagnostic marker in both histopathology and immunohistochemistry. Special stains for histopathological analysis could be a quicker and more affordable approach and can help in faster diagnosis.

**Aims and objectives:** Aim of the study was to compare the staining effectiveness of specific stains (Rapid PAP, AB-PAS, Aldehyde fuschin stain) to H&E stain for keratin.

**Materials and methods**: 80 histopathologically confirmed cases of well-differentiated squamous cell carcinoma (OSCC) were included. 4 micrometer thick sections were cut and stained using special stains - Rapid Papanicolaou (Rapid PAP), Alcian Blue-Periodic Acid Schiff (AB-PAS), Aldehyde Fuschin stain. These were studied under microscope for staining effectiveness. The outcomes were statistically examined.

**Results**: Each of these stains distinguishes keratin with AB-PAS showing better staining quality compared to other two suggesting that it might be used as a substitute stain for keratin.

**Conclusion:** Among special stains the staining capacity of alcian blue- periodic acid Schiff stain was in par with

H&E stain and can be used as an alternative for fast and economical staining along with H&E as compared to immunohistochemistry techniques for rapid diagnosis of OSCC.

**Keywords**: Oral Squamous Cell Carcinoma, Keratin, Hematoxylin & Eosin, Special stains, Diagnosis

**Introduction**

The expression of structural protein keratin preserves the integrity of epithelial tissues and serve as a barrier and protect internal tissues from external bacterial infection, chemical damage and stress[1]. The keratins exhibit a remarkable level of molecular diversity and are the characteristic intermediate filament proteins of epithelia. There are 54 functioning keratin genes in humans[2]. Keratins make up nearly 80% of total protein content in the differentiated layer of stratified epithelia[1]. It also provides mechanical resiliency and structural integrity to eukaryotic cells [3].

Keratin refers to "kera" in Greek which means horn. Intermediate filament-proteins with 10 nm diameter.[4] Hanukoglu and Fuchs described the molecular structure of keratin. Molecular weight is from 44 to 66 kD and these are insoluble in organic solvents and water [5].

Keratins are expressed in pairs as Type II and type I obligatory heterodimer proteins[6]. Chromosome 17 contains the genes for acidic proteins (type l), with the exception of K18; chromosome 12 has the genes for basic proteins (type ll), including K18. A paired dimer is created when a type I chain and its type II counterpart associate in parallel. A staggered tetramer is created when two of these paired dimers join together in an anti-parallel manner. The proto-lament is formed by the lateral packing of these two tetramers. The keratin filament is created by twisting eight of these filaments into a rope. These filaments are then bundled and put together to form macromolecular networks that spread throughout the cytoplasm [7,2].

There are two types of oral epithelium: non-keratinized and keratinized stratified epithelia (ortho and parakeratinized). Abnormalities in keratinization process can lead to many genetic, premalignant and malignant disorders. Keratin proteins are therefore a crucial diagnostic indicator for epithelial diseases [1,8].

Amyloid, collagen, keratin, muscle and other intracellular and extracellular secretions stain eosinophilic in standard hematoxylin and eosin (H&E) staining, making it difficult to distinguish one from another [1,9].

Despite being the "gold standard" for immunohistochemical diagnosis, carcinoma classification and subtyping, and the detection of ambiguous metastases, cytokeratin demonstration is not the favored option because of its expense and time commitment [10]. Hence a less expensive alternative using special stain can be used for early and rapid diagnosis of OSCC.

The keratin pattern can be histologically detected using a variety of specific and fluorescent stains. Keratin can be stained by the Dane-Herman (D H) method) Ayoub-Shklar (A-S) method, Rapid Papanicolaou (Rapid PAP), Congo red, Alcian Blue-Performic Acid Schiff (AB-PAS), Gram stain, Auramine Rhodamine fluorescent method and Aldehyde fuchsin [1,11].

Therefore, using special stain can be a unique, simple, affordable, and time-efficient method. The OSCC cases were treated to the special stains Rapid Papanicolaou (Rapid PAP) stain, Alcian Blue-Periodic Acid Schiff's (AB-PAS), and Aldehyde fuschin stain in this study to check for their staining efficiency.

**Aim and Objectives**

To evaluate the staining effectiveness of specific stains (Rapid PAP, AB-PAS, and aldehyde fuschin stain) and H&E stain for keratin.

**Materials and Methods**

**Study population:** The study included eighty cases of well-differentiated squamous cell carcinoma that were reported to the Department of Oral Pathology & Microbiology. The patients ranged in age from 34 to 70. The study was carried out from September 2024 till November 2025.

**Inclusion and Exclusion criteria:**

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| • Histopathologically confirmed and well differentiated cases of OSCC | • Specimen including any other associated pathology<br>• Treated cases of oral squamous cell carcinoma and oral epithelial dysplasia<br>• Specimen showing less keratin pearls |

**Methods**: 4 micron of paraffin embedded sections were cut and stained with AB-PAS, H&E, Rapid PAP, and Aldehyde fuschin. The standardized protocol from Bancroft and Gamble[12] was followed for the AB-PAS, H&E, Rapid PAP, and Aldehyde Fuschin staining procedures. For every case with every stain, two investigators reported the staining specificity to avoid any bias. Scoring was done based on following criteria.

Criteria chosen for stain assessment as per the scale formulated for our study:

| Criteria | Score |
|---|---|
| Staining Effectiveness | 1- Poor<br>2- Good<br>3- Very Good<br>4- Excellent |

Statistical analysis was done using Chi-square test and kappa value was computed as showed in Table 1

**Results**

All four of the staining techniques used in our investigation—H&E stain, AB-PAS, Rapid PAP, and Aldehyde Fuschin stain—showed unique keratin. However, compared to AB-PAS, Rapid PAP, and Aldehyde Fuschin stain, H&E stained sections had more keratin pearls. AB-PAS was determined to have the best staining efficiency among the special stains, followed by aldehyde fuschin stain and Rapid PAP. H&E stain was shown to have the highest overall staining efficiency out of all unique stains.

Comparison of staining quality among the four staining techniques **(**H & E, AB-PAS, PAP, and Aldehyde Fuchsin**)** showed a statistically significant difference **(**$\chi^2$ = 37.147, p = 0.00002478**).**

Photomicrographs shows oral squamous cell carcinoma stained by H&E (A), AB-PAS (B), Rapid PAP (C) and Aldehyde fuschin stain (D); keratin pearl is marked by arrow

Among the 80 evaluated cases, H & E staining showed the highest proportion of excellent results **(**55.17%**)** followed by very good **(**20%**)** and good **(**8.7%**)** outcomes, with no cases rated as poor. In contrast, AB-PAS staining demonstrated excellent quality in 34.48%**,** very good in

50%**,** good in 13.04%**,** and poor in 28.57% of cases. PAP staining showed a predominance of good results **(**43.48%**)** and very good outcomes **(**80% within its subset, corresponding to 8 cases, 10% of the total**),** while only 3.45% were rated excellent. Aldehyde Fuchsin staining demonstrated the highest proportion of poor results **(**57.14%**)** and good results **(**34.78%**),** with only **6.9%** rated as excellent.

Overall, the findings indicate that H & E staining produced the best overall results**,** with the majority of slides rated as excellent, whereas Aldehyde Fuchsin showed the weakest staining performance**,** reflected by the high percentage of poor ratings. The statistically significant Chi-square value confirms that the quality of staining differs significantly across the four techniques **(**$p < 0.001$**).**

**Discussion**

The oral cavity's epithelium serves as a barrier between the underlying tissues and the oral environment. This is made up of cells that are firmly bonded to one another to form strata. Continuous cell regeneration keeps it intact. It is exposed to various stresses, which necessitates stronger epithelial cells. The development of a keratin surface layer satisfies this need, and the maturation process is known as keratinization [1, 3].

A keratin molecule comprises of a core rod domain of 310 amino acids with an α-helical. Three non-helical linker sequences (L1, L2, and L3) divide the four subdomains (1A, 1B, 2A, and 2B) that make up this central core. The amino and carboxy terminals (H, V, and E end domains) are where keratin filaments differ from one another [1,13,14,15,16,17,]

Because human oral cancer is the sixth most common type of cancer globally due to rising alcohol and tobacco intake as well as related items like betel nut, pan masala, etc., the OSCC cases were selected for the study[1] In these situations, the oral cavity is constantly subjected to different traumas because of the negative effects of chemical, mechanical, and thermal stimuli, which, when combined with inflammatory conditions, may encourage the development of neoplastic alterations. It causes changes in the underlying connective tissue and the oral mucosa, which are reflected in changes in the pattern of keratin expression [1]. In certain situations with scarce epithelial component keratin is a crucial diagnostic marker for grading squamous cell carcinoma and distinguishing between mesenchymal and epithelial malignancies [1].

Johnson and Klein et al. (1956) described using the Papanicolaou dye to show keratin in paraffin-embedded sections [18]. Later, Elzay et al. added phloxine-B, a red acid dye that gives keratin and pre-keratin a noticeable red hue on paraffin-embedded sections, to the standard papanicolaou stain[19]. According to the study, the surface keratin showed up clearly with Rapid PAP AB-PAS and H&E, but not so well with aldehyde fuschin. The H&E stain gave a clear and consistent pattern of keratin, while the other special stains showed a mix of uniform and scattered patterns while the other special stains showed a mix of uniform and scattered patterns.

To show keratin in tissue sections that are fixed in paraffin, Ramalu S. Kale and others (2013) compared a modified PAP stain with Ayoub Shklar and H&E stains. They found that surface keratin is clearly seen with all these stains, but for finding out the exact pattern of cytokeratin in tumours like squamous cell carcinoma, a more sensitive method like immunohistochemistry is needed. This is because some types of keratin in these tumours don’t show up with the modified PAP or Ayoub Shklar stains [9].

Roopa S. Rao and others (2014) compared the usual H&E stain with the Dane-Herman (D-H), Ayoub-Shklar (A-S), Rapid Papanicolaou (Rapid PAP), Alcian Blue-Performic acid Schiff (AB-PAS), and Gram stains in other studies and found that D-H, A-S, and AB-PAS gave staining results similar to H&E. These methods could be used as alternatives to H&E for showing keratin [10].

Keratin pearls that stained well with certain stains might have gone through the same normal process of cornification as regular keratin. This could explain why the number and the way keratin pearls look vary in OSCC cases. On the other hand, keratin pearls that didn't stain might have gone through some unknown changes during cornification. This might make them not react well to certain stains or react less.

Since H&E stain isn't specific to keratin structures and amyloid, collagen, keratin, muscle and other secretions from cells also stain pink making it hard to tell them apart [1].

Further studies on larger sample size and on other special stains can help us to evaluate the staining abilities of these special stains and to use them as an adjuvant to immunohistochemistry for rapid, easy and economical diagnosis of OSCC. Special stains can also be used in areas for diagnosis where immunohistochemistry is not feasible due to geographical or financial restrictions.

**Conclusion**

When looking at the sections, there were more keratin pearls visible in those stained with H&E. Few keratin pearls stained in their center or periphery and few didn't take up special stains at all. This study shows that each of the four stains can detect keratin. All the specific stains clearly marked keratin, which is different from other parts of connective tissue. Out of all the special stains, AB-PAS was found to be the most effective at staining, and it worked as well as the H&E stain. Based on this study, AB-PAS can be used instead of H&E to see keratin. However, H&E is still the best choice for showing keratin in well-differentiated OSCC cases.

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**Legend Figure and Table**

Figure 1 A, B, C, D:

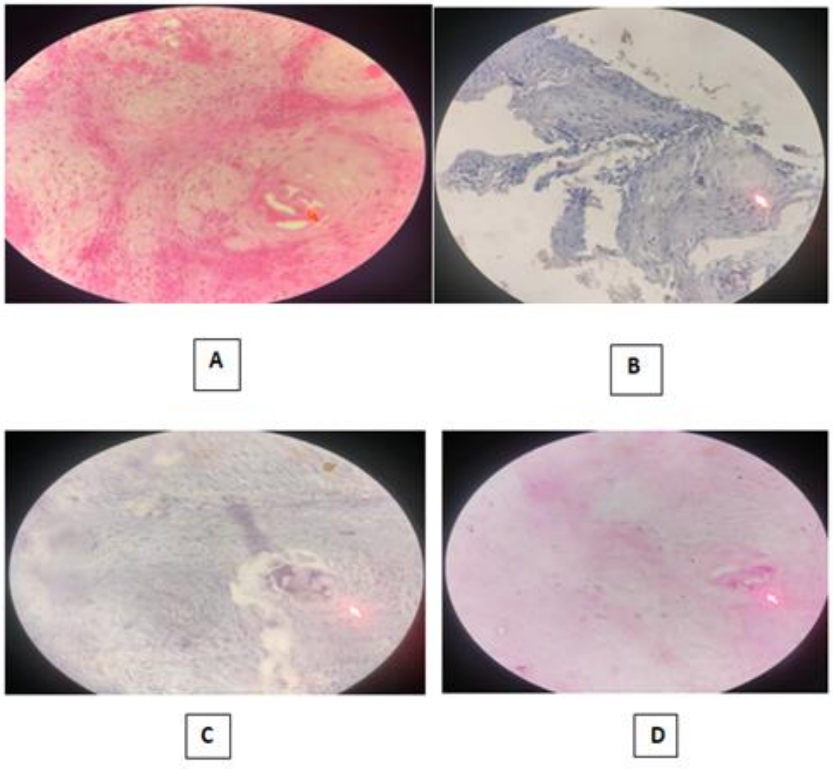

Table 1: The evaluated chi square and p value are respectively 37.147 and 0.00002478

| | Poor | Good | Very Good | Excellent | Total Cases |
|---|---|---|---|---|---|
| H & E | 0 (0%) | 2 (8.7%) | 2 (20%) | 16 (55.17%) | 20 (25%) |
| AB-PAS | 2 (28.57%) | 3 (13.04%) | 5 (50%) | 10 (34.48%) | 20 (25%) |
| PAP | 1 (14.29%) | 10 (43.48%) | 8 (80%) | 1 (3.45%) | 20 (25%) |
| Aldehyde Fuschin | 4 (57.14%) | 8 (34.78%) | 6 (60%) | 2 (6.9%) | 20 (25%) |
| Total | 7 (100%) | 23 (100%) | 21 (210%) | 29 (100%) | 80 (100%) |
| Chi square | 37.147 | | | | |
| P value | 0.00002478 | | | | |