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COMPARATIVE STUDY OF SPECIAL AND HEMATOXYLIN & EOSIN STAINS IN ORAL SQUAMOUS CELL CARCINOMA FOR KERATIN

Pathology

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ABSTRACT

Introduction: Keratins are most abundant structural proteins of cutaneous as well as oral epithelium. A wide range of hereditary and premalignant disorders arise due to defects in the keratinization process. It makes keratin proteins an important diagnostic marker both histopathologically and immunohistochemically. Since immune-histochemistry is an expensive diagnostic technique, the histopathological study with the help of special stains could serve as faster and economic option for visualization of keratin. Moreover, special stains are an integral part of routine histopathology as an adjunct to Hematoxylin and Eosin stain.

Aims and objectives: Aim of the present study was to compare the staining efficacy and staining intensity of special stains (AB-PAS, Rapid PAP and Gram stain with respect to H&E stain for keratin.

Materials and methods: Total number of 30 diagnosed cases of oral lesions of well differentiated squamous cell carcinoma(OSCC), were taken. Four sections of 4 microns thickness each were stained with Alcian Blue-Performic Acid Schiff (AB-PAS), Rapid papanicolaou (Rapid PAP), Gram stain and Hematoxylin & Eosin stain. The results were compared and analyzed statistically.

Results: Keratin is distinguished by all the four stains. AB-PAS demonstrated staining quality comparable to that of H&E stain, indicating it's potential use as alternative stain for keratin.

Conclusion: The keratin is demonstrated by all the four stains. Staining specificity of AB-PAS was found best among all stains and staining intensity was comparable to that of H&E stain.

KEYWORDS

Keratin, Oral Squamous Cell Carcinoma, Hematoxylin & Eosin, Special stains

INTRODUCTION:

Epithelial tissues form a barrier between the body and the environment. It protects the internal tissues from environmental stresses, chemical damage, and bacterial infection.[1]This demand is met by the formation of intracytoplasmic filamentous proteins called keratins which accounts for almost 80% of the total protein content in differentiated cells of stratified epithelia.[2,3,4] They provide structural integrity and mechanical resiliency to all the eukaryotic cells.[5]

The word keratin comes from the Greek word 'kera' meaning horn.[6] Keratins are defined as intermediate filament forming proteins, (10 nm in diameter) with specific physicochemical properties, produced in any vertebrate epithelia.[7] Molecular structure of keratin was described by Hanukoglu and Fuchs. Their molecular weight ranges from 44 to 66 kDa and are extremely insoluble in water and organic solvents.[2]

Keratins are obligate heterodimer proteins, expressed in pairs of type I and II proteins.[8] Genes for acidic proteins (type l), except K18 are found on chromosome 17 while for the basic proteins (type ll), including K18 are found on chromosome 12. Parallel association of a type I chain with its type II counterpart occurs to form a paired dimer. Two such paired dimers associate in an anti-parallel fashion to form a staggered tetramer. These two tetramers pack together laterally to form the proto-filament. Eight such proto-filaments are twisted into a rope to form the keratin filament which are subsequently bundled and assembled into macromolecular networks that radiate throughout the cytoplasm.[9,1]

All keratin molecules contain a central rod domain of 310 amino acids with α-helical conformation. This central core is made up of four sub domains (1A, 1B, 2A, 2B) separated by three non helical linker sequences (L1, L2 and L3). Diversity among keratin filaments resides in the amino and carboxy terminals (H, V and E end domains).[10,11,12,13,14]

The oral epithelium can be keratinized stratified epithelia (ortho and parakeratinized) and non keratinized. Due to defects in the keratinization process large number of hereditary, premalignant and malignant disorders arises. Thus, making keratin proteins an important diagnostic marker in the epithelial pathologies.[15]

In routine hematoxylin and eosin (H&E) staining, structures like collagen, amyloid, muscle, keratin and other extra cellular and intracellular secretions stain eosinophilic, where differentiation of one from another is very difficult.[16]

However demonstration of cytokeratins are the 'gold standard markers' in immunohistochemical diagnosis, classification and sub typing of carcinomas and detection of unclear metastasis but it is not the preferred choice due to its cost and time consumption.[17]

Various special and fluorescent stains can be used to detect the keratin pattern histologically. Ayoub-Shklar (A-S) method, Dane-Herman (D-H) method, Alcian Blue-Performic Acid Schiff (AB-PAS), Rapid papanicolaou (Rapid PAP), Gram stain, Phloxine-tartarizine, Aldehyde fuchsin, Congo red, Thioflavin T and Auramine-Rhodamine fluorescent method can be used to stain keratin.[18] So use of special stain can be specific, easy, cost and time effective alternative In the this study we have subjected the OSCC cases for special stains Alcian Blue-Performic Acid Schiff's (AB-PAS), Rapid papanicolaou (Rapid PAP) stain and Gram stain. These are compared with routine H&E (Hematoxylin & Eosin) stain, with respect to their staining quality for keratin.

AIM AND OBJECTIVES:

- To stain the tissue sections of diagnosed OSCC cases by the special stains: AB–PAS, Rapid PAP and Gram stain.
- To compare the staining specificity and intensity of special stains with respect to routine H&E stain.

MATERIALS AND METHODS:

Study population:-

Thirty cases of well differentiated squamous cell carcinoma which reported to the department of Oral Pathology & Microbiology R.U.H.S. College of Dental Sciences, Jaipur, Rajasthan were included in the study. The patient age group was 30-60 years. The study was conducted between july-2017 to july-2019.

Place of study:- This study was done in the Department of Oral Pathology & Microbiology, R.U.H.S. College of Dental Sciences, Jaipur, Rajasthan.

Methods:- Four sections of each 4 micron thickness were cut from paraffin embedded blocks and subjected to H&E, AB–PAS, Rapid PAP and Gram stain. Staining procedure for H&E, Rapid PAP and Gram stain were followed according to standardized protocol from Bancroft and Gamble.[19] The AB-PAS staining procedure was modified from Scott and Clayton.[20] Acidified permanganate solution was used instead of periodic acid for oxidation. Staining specificity and staining intensity were recorded by two investigators for each case with all stains.

Criteria selected for evaluation of stains.[21]

Criteria	Score
Staining Specificity Staining Intensity	1- Poor 2- Satisfactory 3- Good 4- Excellent

The results were then analyzed statistically using Chi-square test. Kappa value was calculated for inter-observer variations.

Table 1 Staining specificity

Stain	Staining specificity				Total cases	Chi-square value	P value
	1	2	3	4			
H & E	0	4	7	19	30	50.763	0.000
AB-PAS	4	6	8	12	30		
PAP	2	13	15	0	30		
Gram	5	13	12	0	30		

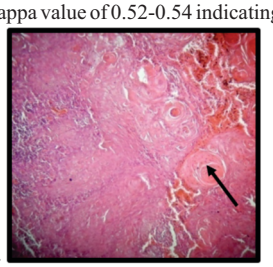
Table 2 Staining intensity

Stain	Staining intensity				Total cases	Chi- square value	P value
	1	2	3	4			
H & E	0	2	6	22	30	59.201	0.000
AB-PAS	2	3	11	14	30		
PAP	1	12	16	1	30		
Gram	1	15	14	0	30		

RESULTS:

In our study all the four staining procedures i.e. H&E stain, AB-PAS, Rapid PAP and Gram stain showed clear distinct keratin. But H&E stained sections showed more amount of keratin pearls than AB-PAS, Rapid PAP and Gram stain. Among the special stains the staining specificity and staining intensity of AB-PAS was found superior and followed by Rapid PAP and Gram stain. However, overall staining specificity and staining intensity of H&E stain was found best in comparison to all the special stains.

Kappa value of 0.52-0.54 indicating fair agreement between observes.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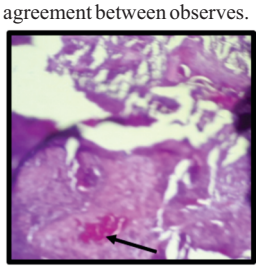
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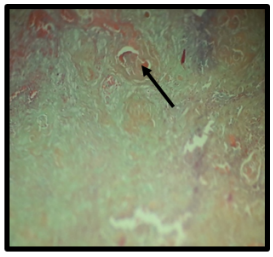
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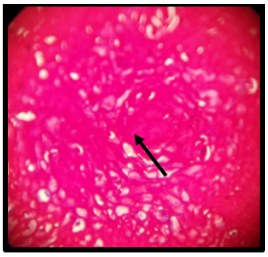
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Sections of oral squamous cell carcinoma stained with H&E (A), AB-PAS (B), Rapid PAP (C) and Gram stain (D); arrow marking keratin pearl

DISCUSSION:

The epithelium of the oral cavity acts as barrier between the oral environment and underlying tissues. This is composed of cells tightly attached to one another and forming strata. Its integrity is maintained by continuous cell renewal. It is subjected to different forms and intensity of stress which demands tougher epithelial cells. This demand is met by the formation of surface layer of keratin and the process of maturation is called keratinization.[5]

The cases of OSCC were chosen for study because human oral cancer is the sixth largest group of malignancies worldwide due to increase in alcohol and tobacco consumption and allied products like betel nut, betel quid, pan masala etc.[22] In these cases, the oral cavity is continually exposed to various traumas due to ill effects of thermal, mechanical and chemical stimuli, which when accompanied by inflammatory states may promote the growth of neoplastic changes. It results in changes in both oral mucosa and the underlying connective tissue, which is reflected in alterations in keratin expression pattern.

Keratin serves as important diagnostic marker in grading of squamous cell carcinoma, to differentiate between the epithelial and mesenchymal tumors and in certain conditions like when the epithelial component may be sparse and may be identified only by the presence of keratin reactivity.

Johnson and Klein *et al*. (1956) reported the application of the papanicolaou stain to paraffin embedded sections, for demonstration of keratin.[23] Later Elzay *et al*. modified the routine papanicolaou stain by adding phloxine-B, which is a red acid dye that stains pre-keratin and keratin distinctly red in color on paraffin embedded sections.[24]

Special stains (Ayoub-Shklar, Dane- Herman, modified PAP, AB-PAS, Gram stain etc.) are used to differentiate specific tissues and cellular structures when applied to histopathological / cytological preparations. These histochemical stains stain keratin specifically and may highlight even small foci of keratinization.[16,17]

In the present study, we compared the special stains (AB-PAS, Rapid PAP and Gram stain) with the routine H&E stain for staining of keratin in the cases of well differentiated squamous cell carcinoma of oral cavity. H&E stain, AB-PAS, Rapid PAP and Gram stain showed clear distinct keratin. The surface keratin was demonstrated good with AB-PAS, Rapid PAP and H&E stain but poorly stained with Gram stain in this study. Pattern of keratin staining was found uniform with H&E stain whereas mixed pattern (uniform and patchy) were seen with all the special stains.

Number of keratin pearls observed were more in H&E stained sections when compared with special stains. Also some keratin pearls stained only in the central core or only periphery of keratin pearl was stained leaving the core unstained with special stains. Even some keratin pearls did not take up the special stain at all.

These findings were consistent with the study done by Ramulu S Kale *et al.* (2013) and Roopa S Rao *et a.l* (2014).

Ramalu S Kale *et al.* (2013) compared modified PAP stain with Ayoub-Shklar and H&E stain for demonstration of keratin in paraffin embedded tissue sections, found that surface keratin is well stained by all of these stains but for the tumor keratin present in squamous cell carcinoma that is not stained by modified PAP and A/S stain, a more sensitive technique like immunohistochemistry be applied to know the exact pattern of cytokeratin expression.[16]

In a similar study Roopa S Rao *et al*. (2014) compared Ayoub-Shklar (A-S) method, Dane-Herman (D-H) method, Alcian Blue-Performic acid Schiff (AB-PAS), Rapid papanicolaou (Rapid PAP) and Gram stain with routine H&E stain, observed that D-H, A-S and AB-PAS demonstrated overall staining quality comparable to H&E, suggestive of their potential use as alternative stains for keratin.[17]

The possible explanation for this difference in amount of keratin pearls and pattern of staining in OSCC cases could be that keratin pearls stained positively with special stains would have undergone normal cornification process as the physiologic keratin. Whereas unstained keratin pearls would have undergone some unknown biochemical differences in the cornification process and thereby have no affinity or less affinity for special stains. Moreover all the keratin pearls seen in H&E stained sections may not be true keratin because H&E stain is not specific for keratin. It stains collagen, amyloid, keratin, muscle and other extracellular and intracellular secretions eosinophilic, so differentiation of one from another is difficult.

CONCLUSION:

The present study shows that the keratin is distinguished by all the four stains. Also, all the special stains distinctly stained keratin distinguishing it from the other connective tissue components. Staining specificity of AB-PAS was found best among all special stains and staining intensity was comparable to that of H&E stain. This study suggests that AB-PAS can be used as alternative stain to H&E for visualization of keratin. But till date H&E stain is still gold standard for demonstration of keratin in well differentiated OSCC cases on an overall basis.

Conflict of interest:- There is no conflict of interest between authors.

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