ORIGINAL RESEARCH PAPER

Volume-7 | Issue-3 | March-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A SHORT STUDY OF KERATIN DEMONSTRATION POTENTIAL OF GIEMSA STAIN, GRAM 'S STAIN AND HAEMATOXYLIN-EOSIN STAIN IN PARAFFIN EMBEDDED TISSUE SECTIONS.

Oral Pathology

Dipak Baliram Patil*

(MDS) Assistant Professor, Department of Dentistry, Walawalkar Rural Medical College and Hospital, Dervan,Chiplun, Maharashtra , India. *Corresponding Author

ABSTRACT

Aims:
1. To stain the known keratin containing tissue by Haematoxylin-Eosin stain,Giemsa stain and Grams stain.
2. To compare efficacy of Giemsa,Grams and Haematoxylin-Eosin staining technique for demonstration of Keratin.

Objective: To device a keratin demonstration procedure which is easy, effective, comparable and advantageous to H-E staining.

Materials and methods: A total 30 paraffin embedded tissue sections of known keratin containing tissue including hyperkeratotic lesions,well differentiated squamous cell carcinoma and odontogenic keratocyst will be taken.

Results: In our study all the three staining showed clear, distinct keratin as pink, Light Pink and Blue respectively.All three stains showed similar results for staining surface keratin in Hyperkeratotic lesions and OKC .

Conclusion: Gram's and Giemsa staining of keratin in Hyperkeratotic lesions and OKC is comparable to staining with H & E. Overall staining efficacy of keratin by H & E in WDSCC is excellent as compared to Grams, Giemsa.

KEYWORDS

Giemsa, Gram, Keratin

INTRODUCTION

Keratins are frequently the most abundant cellular proteins. They constitute the major component of the cytoskeleton of all epithelia; these intermediate filaments provide mechanical support for the cells and nucleus.[1]

Keratins have a number of distinct advantages for use as marker proteins to differentiate epithelial tumors from mesenchymal tumors, both histologically and immunohistochemically.[2]

In establishing a definitive diagnosis, it is sometimes advantageous to demonstrate histologically the degree of keratinization or the presence and / or absence of keratin through the application of a stain which discerns keratin.

In routine hematoxylin and eosin (H-E) staining, structures like collagen, amyloid, muscle, keratin and other extra cellular and intracellular secretions stain eosinophilic where differentiating one from other is difficult. [1]

For example, the histological demonstration of keratin is important in assessing the degree/ pattern of differentiation for squamous cell carcinoma.

In the past, Ayoub-Shklar A-S, Dane - Herman method, modified papanicolaou, Schiff reagent after oxidation with performic acid, aldehyde-fuchsin,levafix red violet have been used to stain keratin.

All these have advantages and disadvantages.

It was Ernst's early observation that keratin stains positively with Gram's Method.

This led us to apply the stain to the keratogenous zones of epithelial structures, and subsequently to attempt a cytochemical analysis of the mechanism of the stain .[3]

Giemsa stain class was originally designed to incorporate cytoplasmic (pink) staining with nuclear (blue) staining and fixation as a single step for smears and thin films of tissues.

Minor modifications of working stain concentration and staining time have been made over the years for fixed tissue sections.[4]

AIMS:

1. To stain the known keratin containing tissue by Haematoxylin-Eosin stain, Giemsa stain and Gram's stain.
2. To compare efficacy of Giemsa, Gram's and Haematoxylin-Eosin staining technique for demonstration of Keratin.

OBJECTIVE:

To device a keratin demonstration procedure which is easy, time saving, effective, comparable and advantageous to H-E staining.

MATERIAL AND METHOD:

Since the study was planned to stain keratin in tissue sections, hematoxylin and eosin stained sections were reviewed for the presence of keratin in known keratin containing tissue sections and slides were selected.

The study group included:

- 10 cases of well differentiated squamous cell carcinoma(WDSCC)
- 10 cases of Odontogenic Keratocyst (OKC).
- 10 cases of Hyperkeratotic lesions.

Three sections of 4 micron thickness paraffin embedded sections were taken.

Sections were stained with
1) Hematoxylin and Eosin
2) Giemsa stains
3) Gram stains, respectively.

Procedure for hematoxylin and eosin:

- The standard H & E staining protocol was followed.

GRAMS Staining Protocol for Tissue Sections: [3-5]

1 Bring sections to distilled water
2. On a rack, flood with filtered crystal violet 30 sec
3. Wash briefly in water to remove excess crystal violet
4. Flood with Gram's iodine 60 sec
5. Wash briefly in water, do not let the section dry out.
6. Decolourise with acetone until the moving dye front has passed the lower edge of the section
7. Wash immediately in tap water
8. Note : If the section appears too blue repeat steps 6 and 7
9. Counterstain with safranin 15 sec
10. Dehydrate absolute alcohol, clear and mount.

GIEMSA'S Staining Protocol for Tissue Sections : [4]

1. Bring sections to distilled water
2. Stain with diluted Freshly made Giemsa's stain at room temperature for 12 to 18 hours.
3. Rinse in distilled water.
4. Differentiate with 0.5% aqueous acetic acid.
5. Dehydrate rapidly
6. Clear and mount

Stained sections were evaluated and the results were analyzed for efficacy of three staining technique and examined for:

1. Amount of keratin present (keratin pearl as per the definition is the central focus of keratinization found within the concentric layers of abnormal squamous cells in epithelial islands).
   a. "More amount of keratin pearl" - more than 3 keratin pearls per field
   b. "Few areas of keratin pearl" - less than 3 keratin pearls per field
2. Pattern of staining (whether uniform or patchy).
3. Time required for Staining.

RESULTS :

In our study all the three staining procedures i.e., H – E stain, Giemsa stain and Gram stain showed clear, distinct keratin as pink, Light Pink and Blue respectively.

All three stains showed similar results for staining surface keratin in Hyperkeratotic lesions and OKC. (Fig 1-6)

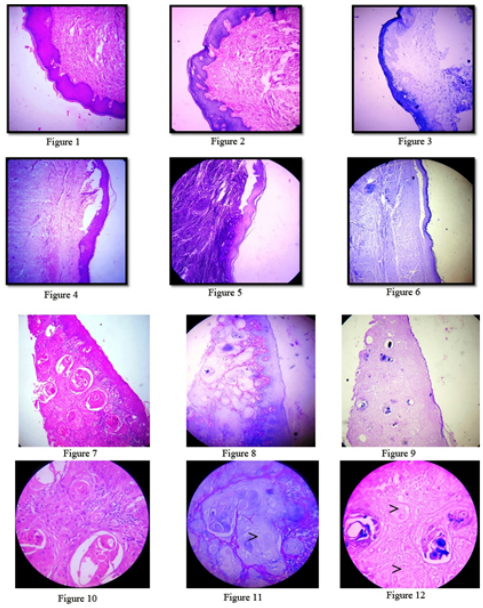
Table 1: Amount of keratin pearls and Pattern of staining in WDSCC

Stain	H & E	Giemsa	Grams
Few	2	7	7
More	8	3	3
Total	10	10	10
Pattern	**H & E**	**Giemsa**	**Grams**
Uniform	10	7	7
Patchy	0	3	3
Total	10	10	10

Table 2: Time required for staining

Stain	H &E	Giemsa	Grams
Time	45 Minutes	16 hours	**15 Minutes**

In our study, in both Giemsa stain and Gram stain only 3 sections of 10 cases of WDSCC showed "more" amount of keratin pearl and 7 section shows 'Few' amount of keratin pearls in both stains. [Table 1] All keratin pearls did not stain uniformly. Some of the keratin pearls stained in the central core or only at the periphery while, in other areas keratin pearl did not take up the special stain at all [Figures 10-12]. Surface epithelium in sections of WDSSC also showed uniformly stained keratin. [Figures 7-9]



DISCUSSION :

Keratin plays an important role as a marker protein in establishing a definitive histological diagnosis, like in grading of squamous cell carcinoma[6], in differentiating epithelial and mesenchymal tumors and in certain conditions when the epithelial component may be sparse and may be identified only by the presence of keratin reactivity.

Literature search revealed keratin staining studies using Ayoub-shklar, Dane Herman method , Modified Papanicolaou [1], Schiff's reagent by oxidation with Performic acid[7], Modified Mallory connective tissue stain [8] ,Haematoxylin-phloxine-alcian blue-orange [9] , levafix red violet e-2b [10], and Gram's stain .

All are special histochemical stains used to study stain keratin specifically. These stains may highlight small foci of overt epithelial differentiation that is sometimes missed in routine H &E staining.

Gram's stain is a routinely used cytopathological stain, available in most of the Oral pathology laboratories.

It was Ernst's early observation that keratin stains positively with Gram's Method .[3]

In 1956 Percy L. Johnson et al did a study on a section of the filliform papillae of the adult cat's tongue by staining with Gram's stain and observed that the most superficial cells of the stratum germinativum show an ever increasing amount of Gram-positive cytoplasm and the superficial keratinized layers of the Papillae stain entirely Gram-positive.[3] Our results coincide with the findings of this study.

To the best of our knowledge, literature search did not reveal any study regarding Gram's staining on paraffin sections for keratin.

Giemsa stain is a member of Romanowski group of stains.

Minor modifications of working stain concentration and staining time have been made over the years for fixed tissue sections.[4]

We claim that ours is a pioneer study since literature review did not reveal any actual study demonstrating keratin using Giemsa stain.

Ramulu et al.[1] compared Modified PAP stain with ayoub-shklar and H& E for demonstration of Keratin in paraffin embeded tissue sections. 'More' amount of keratin pearls were seen in 16/20 ,5/20 and 5/20 cases of WDSCC stained with H &E, Gram's, Giemsa and 'Few' Pearls in 4/20,15/20 and 15/20 cases respectively .

Our results are in accordance with findings of the same study.

In our study, in both Giemsa stain and Gram stain only 3 sections of 10 cases of WDSCC showed "more" amount of keratin pearl and 7 section shows 'Few' amount of keratin pearls in both stains.

All keratin pearls did not stain uniformly. Some of the keratin pearls stained in the central core or only at the periphery while, in other areas keratin pearl did not take up the special stain at all [Figures 10-12].

Surface epithelium in sections of WDSSC also showed uniformly stained keratin.[Figures 7-9] Thus, the efficacy of Gram stain, Giemsa stain is comparable with H &E stain.

Although routine H & E is the gold standard, it is not specific for keratin.

Gram's stain also stains keratin distinctly and rapidly [only 15 min.] As compared to H & E, and is readily available in the laboratory.

Giemsa stains keratin distinctly, also yields nuclear details, uses readily available dyes but is time consuming as compared to H & E.

CONCLUSION:

Our study adds to the limited literature available on application of Gram's,Giemsa stain on paraffin embedded tisssue sections.

Keratin Demonstratin by Gram's and Giemsa stain is Easy, Effective and Comparable to H & E.

Gram's stain is rapid, less time consuming for keratin staining. Grams and Giemsa staining of keratin in Hyperkeratotic lesions and OKC is comparable to staining with H & E. Overall staining efficacy of keratin staining by H & E in WDSCC is excellent as compared to Grams , Giemsa.

REFERENCES

1. Surekha Ramulu et al. Comparing modified papanicolaou stain with ayoub-shklar and haematoxylin-eosin stain for demonstration of keratin in paraffin embedded tissue sections. JOMFP Vol. 17 Issue 1; 2013:23-30.
2. Shibani Shetty et al. Keratinization and its Disorders. Oman Medical Journal (2012) Vol. 27, No. 5: 348-357.

3. Percy L. Johnson et al. The gram staining mechanism of cat tongue keratin. J Histochem Cytochem 1957 5: 84
4. Lillie, R.D. , Histopathologic Technique and Practical Histochemistry, 3rd edition, McGraw-Hill;1965.
5. Lillie RD,The Gram Stain. A quick method for staining gram positive organisms in tissue. Arch Path., 5;1977:828-834.
6. Elzay RB et al.A modification of the Papanicolaou exfoliative cytology stain to demonstrate keratin in paraffin-block tissue sections. Oral Surg Oral Med Oral Patho.1983 Jul; 56(1):51-3.
7. Scott HR. Demonstration of keratin with aldehyde-fuchsin.Nature 1953; 172:674-5.
8. AYOUB P ET AL. A modification of the Mallory connective tissue stain as a stain for keratin. Oral Surg Oral Med Oral Pathol. 1963 May; 16:580-1.
9. DANE et.al.Haematoxylin-phloxine-Alcian blue-orange G differential staining of prekeratin, keratin and mucin., HERMAN DL Stain technology 38: 1963 Mar :97-101
10. Waldrop FS et.al. Staining of keratin and keratohyalin with the reactive dye levafix red violet E-2BL. Stain Techno. 1976 Jul; 51(4):219-25.