Importance of Special Stains As An Aid In Histopathological Diagnosis Of Oral Lesions – A Short Review

SAI SUDHA MAHAJAN1, GHEENA SUKUMARAN2*, PRATIBHA RAMANI3, ABILASHA RAMASUBRAMANIAN4, HANNAH. R5

1Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India.
2Associate Professor,Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India.
3Professor and Head of Department of Oral Pathology,Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India.
4Reader,Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India.
5Senior lecturer,Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India.

*Corresponding Author
Email :151807005.sdc@saveetha.com1, gheena@saveetha.com2, pratibaramani@saveetha.com3
abilasha@saveetha.com4, hannahr.sdc@saveetha.com5

Abstract: In histopathology, special stains help to enhance and assist with diagnosis. Special stains are usually performed after initial examination by H&E stained sections from a repeat cut-section on a paraffin block. Special staining technique provides valuable information in the evaluation of numerous abnormal or disease conditions. Therefore, this review focused to analyse the importance of special stains as an aid in diagnosis. Articles were searched in online databases, such as PUBMED, MEDLINE and Google scholar with keywords “oral lesions, special stains, histopathological diagnosis, importance.” Manual Search of Journals were also done. Articles were reviewed and analysed. The 3 relevant articles were reviewed which showed substantial importance of special stains in oral lesion biopsies. Special stains reveal the nature of keratin, collagen & elastic fibres, basal lamina in epithelium & tumor islands in oral Malignancies. Therefore, in modern pathology the role of special stains are helpful in detecting many oral lesions characteristic of pathologies, making it an important diagnostic tool.

Keywords: Special stains; Histopathology; Diagnosis

INTRODUCTION
Special stains are not routine but they are used as alternative stains which require advanced staining techniques that are performed to visualise selected tissue elements, entities and microorganisms. Special stains satisfactorily demonstrate the tissue components or organisms in tissue sections of interest. Special stains are sometimes utilised in a diagnostic setting, including their indications and the clinical situations ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezhilarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) where they might be useful to reach a diagnosis. ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezhilarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019); (Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezhilarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) The haematoxylin and eosin (H&E) stain is the routine stain used everyday and commonly to stain histological sections ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezhilarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) Special stains are sometimes utilised in a diagnostic setting, including their indications and the clinical situations. This is an Open Access Article distributed under the CC BY license. (http://creativecommons.org/licenses/by/4.0/)
Based on the appearance of the initial H&E section, a pathologist may request further sections of ‘special’ stains for a number of reasons to identify a particular tissue structure that does not stain well with routine H&E staining and those which are most abundantly ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) preferred during a diagnostic dilemma. ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) of oral pathologies. (Bancroft, 2008; Kumar and Kiernan, 2010) Special stains hold promise which showed evidence that certain studies have been done to correlate the clinical ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) and histopathological ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) significance which aids in the diagnosis of oral lesions. ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) of oral pathologies. (Bancroft, 2008; Kumar and Kiernan, 2010) Special stains hold promise which showed evidence that certain studies have been done to correlate the clinical ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) and histopathological ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) significance which aids in the diagnosis of oral lesions. ((Gifrina Jayaraj, Ramani, et al., 2015; Gifrina Jayaraj, Sherlin, et al., 2015; G. Jayaraj et al., 2015; Jangid et al., 2015; Sherlin et al., 2015; Sivaramakrishnan and Ramani, 2015; Swathy, Gheena and Varsha, 2015; Gupta and Ramani, 2016; Thangaraj et al., 2016; Viveka et al., 2016; Sridharan, Ramani and Patankar, 2017; Hannah et al., 2018; Gheena and Ezharlarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019)) Our team has rich experience in research and we have collaborated with numerous authors over various topics in the past decade (Deogade, Gupta and Ariga, 2018; Ezharlarasan, 2018; Ezharlarasan, Sokal and Najimi, 2018; Jeevanand and Govindaraju, 2018; J et al., 2018; Menon et al., 2018; Prabakar et al., 2018; Rajeshkumar et al., 2018, 2019; Vishnu Prasad et al., 2018, 2019; Wahab et al., 2018; Dua et al., 2019; Duraisamy et al., 2019; Ezharlarasan, Apoorva and Ashok Vardhan, 2019; Gheena and Ezharlarasan, 2019; Malli Sureshbabu et al., 2019; Mehta et al., 2019; Panchal, Jeevanand and Subramanian, 2019; Rajendran et al., 2019; Ramakrishnan, Dhanalakshmi and Subramanian, 2019; Sharma et al., 2019; Varghese, Ramesh and Veeraiyyan, 2019; Gomathi et al., 2020; Samuel, Acharya and Rao, 2020) This review focused on analysing the importance of special stains as an aid in diagnosis.

**MATERIALS AND METHODS**

**Search strategy for identification of studies:** The search strategy was in accordance with the Cochrane guidelines for systematic reviews. Articles were searched and selected using PUBMED, MEDLINE and Google scholar. Owing to scarcity of importance of special stains in diagnosis of oral lesion biopsies, all possible studies were included. The article search included only those published in the English literature. An Internet search was also done with the key words “oral lesions, special stains, histopathological diagnosis, importance.” Literatures

---

also evaluating the ‘importance of special stains as an aid in Histopathological diagnosis of oral lesions’ in cross-references were also included.

Selection criteria
The title of the articles and abstracts was reviewed. Articles that considered histopathological diagnosis only on oral biopsy basis with the use of special stains were selected for further appraisal.

Data extraction and analysis
Once a final conclusion was arrived at regarding the articles to be reviewed, data extracted from each article were tabulated and was later cross checked. (Table 1) Flow chart for study selection. (fig. 1)

Outcomes
The biopsies of oral lesion in histopathological diagnosis using special stains were analysed.

RESULTS AND DISCUSSION
Methods of review
The selection and exclusion criteria of the reviewed studies are shown in Fig. 1. The search strategy identified three studies that evaluated histopathological diagnosis of oral lesions using special stains. The descriptions of the individual studies are shown in (Table 1).

Included studies
Among the three included studies, the importance of special stains were studied in detail on the Histopathological diagnosis of various oral lesions. (Table 1) One study included the special stains used in the cytologic diagnosis of oral lesion. Another study showed evidence on the importance of special stains used for salivary mucins in normal and neoplastic salivary glands. Third study evaluated special stains used in the diagnosis of oral lesions associated with oral epithelial dysplasia.

Outcomes
The importance of special stains which aided in histopathological diagnosis of oral lesions was assessed in all the three studies, which showed substantial relevance.

Each diagnostic hypothesis of oral lesions may totally depend on the differential stains being characteristic of specific oral pathology. In regard to the report by Almeida JD, et al., differential staining quality of cytoplasmic and nuclear cellular morphologic characteristics and the identification of bacteria, fungi, inflammatory cells and secretions. The results showed that Papanicolaou staining is the best method for cytologic diagnosis of oral lesions and that in cases of fungal diseases such as candidiasis and paracoccidioidomycosis, PAS staining is useful for a better demonstration of the fungus. It is a multichromatic staining technique, which can be used to differentiate cells in smear preparations for various body secretions. (Salati et al., no date; Almeida et al., 2008)

Further, in regard to oral epithelial dysplasia differential staining, the main use of orange G6 in the PAP stain may be used to demonstrate keratin. Nasir A. Salat et al have reported the modification of papanicolaou stain by adding phloxine-B on paraffin embedded sections to demonstrate keratin. Phloxine-B, a red acid dye is a derivative of fluorescein with distinctly bluish shade stains mucin, prekeratin and also epithelial keratin which appear distinctly red in colour. Leishman staining may be used to demonstrate SCC and viral infections like herpes simplex whereas PAP proved to be an ineffective staining technique in the study reported by Almeida JD, et al. Nasir A. Salat et al have reported Toluidine stain (TB) to be used as a vital stain to highlight potentially malignant oral lesions and identify early lesions, which could be missed out on clinical examination. TB stains are useful in obtaining the marginal control of carcinoma and also selecting the biopsy sample site in premalignant lesions. The demonstration of loss of heterozygosity may be detected in TB-stained lesions. Which stains the tissue dark royal blue or pale royal blue color. In diagnosis TB is also widely used to highlight mast cell granules. (Salati et al., no date; Almeida et al., 2008)

In the study by Sushma Naag et al, reported histochemical staining techniques used for normal salivary glands, the serous acini showed negative by all stains except AB stain. The mucous cells of sublingual and submandibular glands (mixed) with diverse heterogeneity in a single acinus and in between clusters of acini, thus indicating a mixture of both acid and neutral mucins coexistence in the same gland. The chondromyxoid areas of pleomorphic adenoma also expressed strong positivity with the AB, PAS and MC stains. (Figure. 2-4) The staining pattern of ADCC with PAS, MC, AB and AF staining on the pseudocystic spaces, concluded that apart from PAS, MC and AB positivity; there was a strong AF positivity. The mucous cells and the lumen of MEC revealed positive staining to MC, PAS and AF staining, thus indicating the presence of sulphated mucins. The AF-AB staining also indicated the presence of sulpho and sialomucins in those areas. The stroma revealed a dominance of sialomucins when the AF-AB staining was employed. (Figure. 4-7) The epidermoid cells stained
negative to all stains. The biochemical and histochemical analysis of salivary glands revealed the presence of mucins as the main component, which showed sugar moieties and amino acids. Hence the histochemical properties of mucins showed the presence of glycoproteins and proteoglycans which differed in their chemical and structural natures. Mucins showed alterations of their normal and pathological states using special stains like Periodic Acid Schiff Reagent [PAS], Alcian Blue [AB], Aldehyde Fuchsin [AF], Mucicarmine [MC] which were categorized into acidic, neutral, sulpho and sialomucins. (Naag and Adi, 2010)

CONCLUSION

Special stains reveal the nature of keratin, collagen & elastic fibres, basal lamina in epithelium & tumor islands in oral Malignancies. Therefore, in modern pathology the role of special stains are helpful in detecting many oral pathologies and the characteristics, making it an important diagnostic tool.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the help offered by the Department of Oral Pathology and Microbiology and the management of Saveetha dental college and hospital for their support.

AUTHORS CONTRIBUTIONS

Author 1 (Dr. Sai Sudha Mahajan), carried out the systematic review by collecting articles and drafted the manuscript after performing the necessary search strategy. Author 2 (Dr. S. Gheena) aided in the conception of this topic and supervised in the preparation of the manuscript. Author 3 (Dr. Pratibha Ramani) has coordinated in developing the manuscript. All the authors have discussed the results among themselves and contributed to the final manuscript.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

REFERENCES

37. Salati, N. A. et al. (no date) ‘DIAGNOSTIC & PROGNOSTIC POTENTIAL OF SPECIAL STAINS IN ORAL EPITHELIAL DYSPLASIA-A CLINICOPATHOLOGIC STUDY’. Available at: https://www.ama.ac.in/pdf/dentjour/DIAGNOSTIC%20&%20PROGNOSTIC.pdf.
Importance of Special Stains as an Aid in Histopathological Diagnosis of Oral Lesions – A Short Review

Fig. 1: shows flow chart for study selection relating to the Importance of Special Stains as an aid in Histopathological Diagnosis of Oral Lesions

Table 1: shows description of included studies

<table>
<thead>
<tr>
<th>S.NO</th>
<th>CITATIONS</th>
<th>SPECIAL STAINS</th>
<th>ORAL LESIONS</th>
<th>HISTOPATHOLOGICAL DIAGNOSIS</th>
<th>INFERENCES</th>
<th>ADVANTAGES / LIMITATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Janete Dias Almeida, et al., 2008</td>
<td>Pap, PAS, Leishman stain</td>
<td>Cytologic Diagnosis of Oral Lesions</td>
<td>erythematous candidiasis (n=9), pseudomembranous candidiasis (n=10), SCC (n=19), herpes simplex (n=8), paracoccidioidomycosis (n=8) and pemphigus vulgaris (n=1)</td>
<td>The cytologic diagnosis of oral lesions allied with different staining methods is a useful tool for oral diagnosis.</td>
<td>The Leishman and PAS techniques did not permit a clear demonstration of cell morphology.</td>
</tr>
<tr>
<td>2.</td>
<td>Sushma Nag, Ravi Prakash Adi., 2010</td>
<td>PAS, Alcian blue (AB), Aldehyde fuchsin (AF), Mucicarmin e (MC)</td>
<td>Salivary Mucins In Normal And Neoplastic Salivary Gland</td>
<td>19 salivary gland neoplasms - 9 pleomorphic adenoma, 4 adenoid cystic carcinoma and 6 mucoepidermoid carcinoma and 10 normal salivary glands (1 parotid, 7 submandibular, 2 sublingual)</td>
<td>The results showed varied heterogeneity of mucin expression in mucous acini and change in mucin expression from benign to malignancy.</td>
<td>Studies on the mucin histochemistry of salivary gland tumours are very few and are not amply reviewed. The nature or content of these mucins are not well documented. The classification of salivary gland tumours on the nature of their mucin content has not been done till date, unlike the classifications of gastrointestinal diseases.</td>
</tr>
<tr>
<td>3.</td>
<td>Nasir A. Salati et al, 2018</td>
<td>Modified Pap, PAS,</td>
<td>Oral lesions associated</td>
<td>100 paraffin embedded tissue</td>
<td>Special stains reveal the nature</td>
<td>Various special stains reveal the</td>
</tr>
<tr>
<td>Van Giesons, Toluidine stain</td>
<td>sections, 10 of normal mucosa, 30 each of mild dysplasia, moderate dysplasia &amp; severe dysplasia</td>
<td>of keratin, collagen &amp; elastic fibres, basal lamina in epithelium &amp; tumor islands in oral premalignant disorders and other lesions which have dysplastic features in epithelium.</td>
<td>integrity of basal lamina in Oral Potentially Malignant Disorders.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2**: Photomicrograph showing ductal lumen and chondromyxoid areas of PA in H&E (10X). [Courtesy: Article source (Naag and Adi, 2010)]

**Fig. 3**: Photomicrograph showing ductal lumen and chondromyxoid areas of PA in MC (10X). [Courtesy: Article source (Naag and Adi, 2010)]
Fig. 4: Photomicrograph showing ductal lumen and chondromyxoid areas of PA in AF-AB (10X). [Courtesy: Article source (Naag and Adi, 2010)]

Fig. 5: Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in H&E (10X). [Courtesy: Article source (Naag and Adi, 2010)]
Fig. 6: Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in MC (10X). [Courtesy: Article source (Naag and Adi, 2010)]

Fig. 7: Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in AF-AB (10X). [Courtesy: Article source (Naag and Adi, 2010)]

TABLES AND FIGURES
Figure- 1: shows flow chart for study selection
Table- 1: shows description of included studies
Figure- 2 : Photomicrograph showing ductal lumen and chondromyxoid areas of PA in H&E (10X) [Courtesy: Article source (Naag and Adi, 2010)]
Figure- 3 : Photomicrograph showing ductal lumen and chondromyxoid areas of PA in MC (10X) [Courtesy: Article source (Naag and Adi, 2010)]
Figure- 5 : Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in H&E (10X) [Courtesy: Article source (Naag and Adi, 2010)]
Figure- 6 : Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in MC (10X) [Courtesy: Article source (Naag and Adi, 2010)]
Figure- 7 : Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in AF-AB (10X) [Courtesy: Article source (Naag and Adi, 2010)]