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

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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)) and is the first section on a slide a pathologist looks at for a case. ((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)) Haematoxylin colours nuclei and a few other tissue elements blue commonly referred to as 'basophilic', while the eosin counterstains the cytoplasm in various shades of pink generally termed 'pale' to 'brightly' to 'deeply eosinophilic' depending on the precise shade. (Dobromylskyj and Others, 2014)

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 Ezhilarasan, 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 Ezhilarasan, 2019a; Hema Shree et al., 2019; Sridharan et al., 2019))

An exhaustive list of special stains are available for use, this review would concentrate on the list of special stains that commonly aid in the histopathologic 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) ; (Sridhar et al., 2016)) 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 Ezhilarasan, 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 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)) 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 Ezhilarasan, 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; Ezhilarasan, 2018; Ezhilarasan, Sokal and Najimi, 2018; Jeevanandan 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; Wahab et al., 2018; Dua et al., 2019; Duraisamy et al., 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Gheena and Ezhilarasan, 2019b; Malli Sureshbabu et al., 2019; Mehta et al., 2019; Panchal, Jeevanandan and Subramanian, 2019; Rajendran et al., 2019; Ramakrishnan, Dhanalakshmi and Subramanian, 2019; Sharma et al., 2019; Varghese, Ramesh and Veeraiyan, 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 guide-lines 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)Our institution is passionate about high quality evidence based research and has excelled in various fields ((Pc, Marimuthu and Devadoss, 2018; Ramesh et al., 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Ramadurai et al., 2019; Sridharan et al., 2019; Vijayashree Priyadharsini, 2019; Mathew et al., 2020)

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.

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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

1. Almeida, J. D. et al. (2008) ‘Evaluation of staining methods for cytologic diagnosis of oral lesions’, Acta cytologica, 52(6), pp. 697–701.
2. Bancroft, J. D. (2008) Theory and Practice of Histological Techniques. Elsevier Health Sciences.
3. Deogade, S., Gupta, P. and Ariga, P. (2018) ‘Effect of monopoly-coating agent on the surface roughness of a tissue conditioner subjected to cleansing and disinfection: A Contact Profilometric In vitro study’, Contemporary Clinical Dentistry, p. 122. doi: 10.4103/ccd.ccd_112_18.
4. Dobromylskyj, M. and Others (2014) ‘Understanding special stains’, Veterinary Times, 44(15), pp. 11–15.
5. Dua, K. et al. (2019) ‘The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress’, Drug development research, 80(6), pp. 714–730.
6. Duraisamy, R. et al. (2019) ‘Compatibility of Nonoriginal Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Nonoriginal Abutments’, Implant dentistry, 28(3), pp. 289–295.
7. Ezhilarasan, D. (2018) ‘Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective’, Arab journal of gastroenterology: the official publication of the Pan-Arab Association of Gastroenterology, 19(2), pp. 56–64.
8. Ezhilarasan, D., Apoorva, V. S. and Ashok Vardhan, N. (2019) ‘Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells’, Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology, 48(2), pp. 115–121.
9. Ezhilarasan, D., Sokal, E. and Najimi, M. (2018) ‘Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets’, Hepatobiliary & pancreatic diseases international: HBPD INT, 17(3), pp. 192–197.
10. Gheena, S. and Ezhilarasan, D. (2019a) ‘Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells’, Human & Experimental Toxicology, pp. 694–702. doi: 10.1177/0960327119839173.
11. Gheena, S. and Ezhilarasan, D. (2019b) ‘Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells’, Human & experimental toxicology, 38(6), pp. 694–702.
12. Gomathi, A. C. et al. (2020) ‘Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line’, Journal of Drug Delivery Science and Technology, p. 101376. doi: 10.1016/j.jddst.2019.101376.

13. Gupta, V. and Ramani, P. (2016) 'Histologic and immunohistochemical evaluation of mirror image biopsies in oral squamous cell carcinoma', Journal of Oral Biology and Craniofacial Research, 6(3), pp. 194–197.
14. Hannah, R. et al. (2018) 'Awareness about the use, ethics and scope of dental photography among undergraduate dental students dentist behind the lens', Research Journal of Pharmacy and Technology, 11(3), pp. 1012–1016.
15. Hema Shree, K. et al. (2019) 'Saliva as a Diagnostic Tool in Oral Squamous Cell Carcinoma - a Systematic Review with Meta Analysis', Pathology oncology research: POR, 25(2), pp. 447–453.
16. Jangid, K. et al. (2015) 'Ankyloglossia with cleft lip: A rare case report', Journal of Indian Society of Periodontology, 19(6), pp. 690–693.
17. Jayaraj, G., Sherlin, H. J., et al. (2015) 'Cytomegalovirus and Mucoepidermoid carcinoma: A possible causal relationship? A pilot study', Journal of oral and maxillofacial pathology: JOMFP, 19(3), pp. 319–324.
18. Jayaraj, G., Ramani, P., et al. (2015) 'Inter-observer agreement in grading oral epithelial dysplasia – A systematic review', Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology, pp. 112–116. doi: 10.1016/j.ajoms.2014.01.006.
19. Jayaraj, G. et al. (2015) 'Stromal myofibroblasts in oral squamous cell carcinoma and potentially malignant disorders', Indian journal of cancer, 52(1), pp. 87–92.
20. Jeevanandan, G. and Govindaraju, L. (2018) 'Clinical comparison of Kedo-S paediatric rotary files vs manual instrumentation for root canal preparation in primary molars: a double blinded randomised clinical trial', European Archives of Paediatric Dentistry, pp. 273–278. doi: 10.1007/s40368-018-0356-6.
21. J, P. C. et al. (2018) 'Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study', Clinical implant dentistry and related research, 20(4), pp. 531–534.
22. Kumar, G. L. and Kiernan, J. A. (2010) 'Special stains and H&E', Dako North America.
23. Malli Sureshbabu, N. et al. (2019) 'Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases', Case reports in dentistry, 2019, p. 7046203.
24. Mathew, M. G. et al. (2020) 'Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary …', Clinical oral investigations. Available at: https://link.springer.com/article/10.1007/s00784-020-03204-9.
25. Mehta, M. et al. (2019) 'Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases', Chemico-biological interactions, 308, pp. 206–215.
26. Menon, S. et al. (2018) 'Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism', Colloids and Surfaces B: Biointerfaces, pp. 280–292. doi: 10.1016/j.colsurfb.2018.06.006.
27. Naag, S. and Adi, R. P. (2010) 'Histochemical study of salivary mucins in normal and neoplastic salivary glands', Journal of clinical and diagnostic research: JCDR. Available at: https://www.researchgate.net/profile/Adi_Prakash/publication/304351586_JOURNAL_OF_CLINICAL_AND_DIAGNOSTIC_RESEARCH_Histochemical_Study_Of_Salivary_Mucins_In_Normal_And_Neoplastic_Salivary_Glands/links/576cbf1408ae193ef3a9ac6b.pdf.
28. Panchal, V., Jeevanandan, G. and Subramanian, E. M. G. (2019) 'Comparison of post-operative pain after root canal instrumentation with hand K-files, H-files and rotary Kedo-S files in primary teeth: a randomised clinical trial', European archives of paediatric dentistry: official journal of the European Academy of Paediatric Dentistry, 20(5), pp. 467–472.
29. Pc, J., Marimuthu, T. and Devadoss, P. (2018) 'Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study', Clinical implant dentistry and related research. Available at: https://europepmc.org/article/med/29624863.
30. Prabakar, J. et al. (2018) 'Comparative Evaluation of Retention, Cariostatic Effect and Discoloration of Conventional and Hydrophilic Sealants - A Single Blinded Randomized Split Mouth Clinical Trial', Contemporary clinical dentistry, 9(Suppl 2), pp. S233–S239.
31. Rajendran, R. et al. (2019) 'Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study', Pesquisa Brasileira em Odontopediatria e Clínica Integrada, pp. 1–10. doi: 10.4034/pboci.2019.191.61.
32. Rajeshkumar, S. et al. (2018) 'Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells', Enzyme and microbial technology, 117, pp. 91–95.
33. Rajeshkumar, S. et al. (2019) 'Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through Cissus arnotiana plant extract', Journal of photochemistry and photobiology. B, Biology, 197, p. 111531.
34. Ramadurai, N. et al. (2019) 'Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial', Clinical oral investigations, 23(9), pp. 3543–3550.

35. Ramakrishnan, M., Dhanalakshmi, R. and Subramanian, E. M. G. (2019) 'Survival rate of different fixed posterior space maintainers used in Paediatric Dentistry - A systematic review', The Saudi dental journal, 31(2), pp. 165–172.
36. Ramesh, A. et al. (2018) 'Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study', Journal of periodontology, 89(10), pp. 1241–1248.
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.amu.ac.in/pdf/dentjour/DIAGNOSTIC%20&%20PROGNOSTIC.pdf.
38. Samuel, S. R., Acharya, S. and Rao, J. C. (2020) 'School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial', Journal of public health dentistry, 80(1), pp. 51–60.
39. Sharma, P. et al. (2019) 'Emerging trends in the novel drug delivery approaches for the treatment of lung cancer', Chemico-biological interactions, 309, p. 108720.
40. Sherlin, H. et al. (2015) 'Expression of CD 68, CD 45 and human leukocyte antigen-DR in central and peripheral giant cell granuloma, giant cell tumor of long bones, and tuberculous granuloma: An immunohistochemical study', Indian Journal of Dental Research, p. 295. doi: 10.4103/0970-9290.162872.
41. Sivaramakrishnan, S. M. and Ramani, P. (2015) 'Study on the Prevalence of Eruption Status of Third Molars in South Indian Population', Biology and Medicine. doi: 10.4172/0974-8369.1000245.
42. Sridharan, G. et al. (2019) 'Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma', Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology, 48(4), pp. 299–306.
43. Sridharan, G., Ramani, P. and Patankar, S. (2017) 'Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma', Journal of cancer research and therapeutics, 13(3), pp. 556–561.
44. Sridhar, C. et al. (2016) 'Evaluation of Habit Patterns and Clinical Findings of Oral Submucous Fibrosis in South Indian Population', Journal of pharmaceutical and biomedical sciences, 6(7). Available at: http://lawarencepress.com/ojs/index.php/JPBMS/article/view/292 (Accessed: 13 June 2020).
45. Swathy, S., Gheena, S. and Varsha, S. L. (2015) 'Prevalence of pulp stones in patients with history of cardiac diseases', Research Journal of Pharmacy and Technology, 8(12), pp. 1625–1628.
46. Thangaraj, S. V. et al. (2016) 'Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations', PloS one, 11(6), p. e0156582.
47. Varghese, S. S., Ramesh, A. and Veeraiyan, D. N. (2019) 'Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students', Journal of dental education, 83(4), pp. 445–450.
48. Vijayashree Priyadharsini, J. (2019) 'In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens', Journal of periodontology, 90(12), pp. 1441–1448.
49. Vishnu Prasad, S. et al. (2018) 'Report on oral health status and treatment needs of 5-15 years old children with sensory deficits in Chennai, India', Special care in dentistry: official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry, 38(1), pp. 58–59.
50. Viveka, T. S. et al. (2016) 'p53 Expression Helps Identify High Risk Oral Tongue Pre- malignant Lesions and Correlates with Patterns of Invasive Tumour Front and Tumour Depth in Oral Tongue Squamous Cell Carcinoma Cases', Asian Pacific journal of cancer prevention: APJCP, 17(1), pp. 189–195.
51. Wahab, P. U. A. et al. (2018) 'Scalpel Versus Diathermy in Wound Healing After Mucosal Incisions: A Split-Mouth Study', Journal of oral and maxillofacial surgery: official journal of the American Association of Oral and Maxillofacial Surgeons, 76(6), pp. 1160–1164.

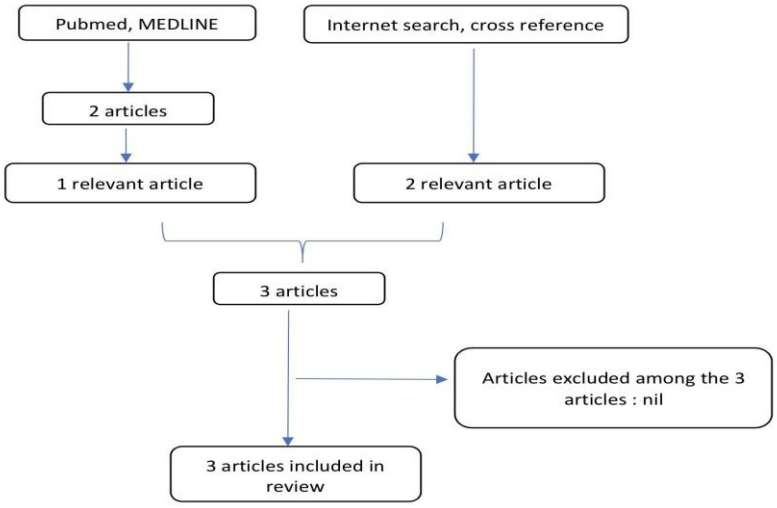

Fig.1: shows flow chart for study selection relating to the Importance of Special Stains as an aid in Histopathological Diagnosis of Oral Lesions

Table 1: shows description of included studies

S.NO	CITATIONS	SPECIAL STAINS	ORAL LESIONS	HISTOPATHOLOGICAL DIAGNOSIS	INFERENCES	ADVANTAGES / LIMITATIONS
1.	Janete Dias Almeida, et al., 2008	Pap, PAS, Leishman stain	Cytologic Diagnosis of Oral Lesions	erythematous candidiasis (n=9), pseudomembranous candidiasis (n=10), SCC (n=19), herpes simplex (n=8), paracoccidioidomycosis (n=8) and pemphigus vulgaris (n=1)	The cytologic diagnosis of oral lesions allied with different staining methods is a useful tool for oral diagnosis.	The Leishman and PAS techniques did not permit a clear demonstration of cell morphology.
2.	Sushma Nag, Ravi Prakash Adi., 2010	PAS, Alcian blue (AB), Aldehyde fuchsin (AF), Mucicarmine (MC)	Salivary Mucins In Normal And Neoplastic Salivary Gland	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)	The results showed varied heterogeneity of mucin expression in mucous acini and change in mucin expression from benign to malignancy.	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.
3.	Nasir A. Salati et al, 2018	Modified Pap, PAS,	Oral lesions associated	100 paraffin embedded tissue	Special stains reveal the nature	Various special stains reveal the

		Van Giesons, Toluidine stain	with epithelial dysplasia	sections, 10 of normal mucosa, 30 each of mild dysplasia, moderate dysplasia & severe dysplasia	of keratin, collagen & elastic fibres, basal lamina in epithelium & tumor islands in oral premalignant disorders and other lesions which have dysplastic features in epithelium.	integrity of basal lamina in Oral Potentially Malignant Disorders.

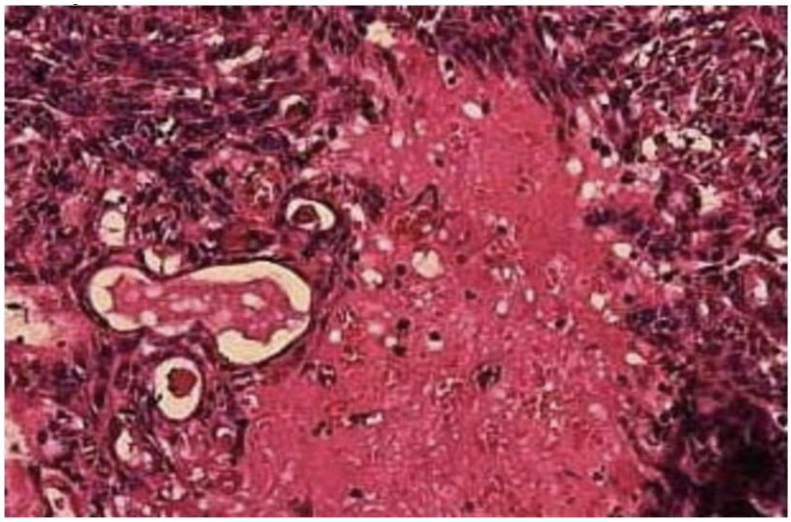

Fig.2 : Photomicrograph showing ductal lumen and chondromyxoid areas of PA in H&E (10X). [Courtesy: Article source (Naag and Adi, 2010)]

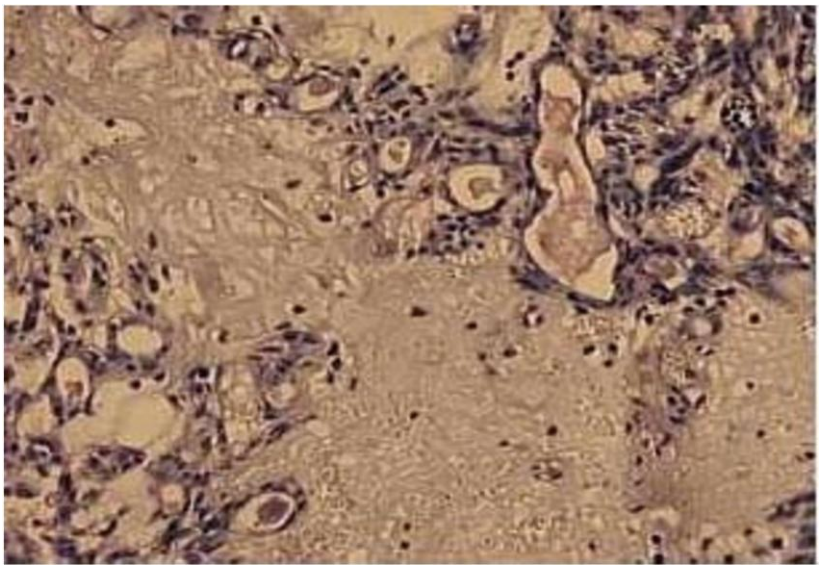

Fig.3 : Photomicrograph showing ductal lumen and chondromyxoid areas of PA in MC (10X). [Courtesy: Article source (Naag and Adi, 2010)]

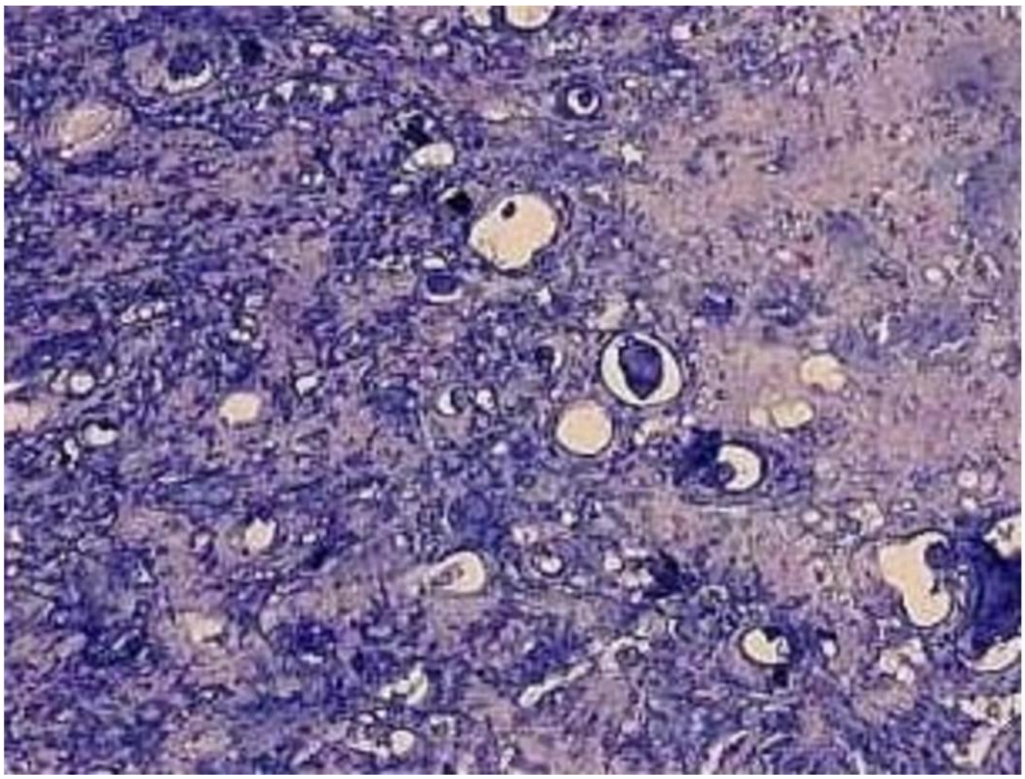

Fig.4 : Photomicrograph showing ductal lumen and chondromyxoid areas of PA in AF-AB (10X). [Courtesy: Article source (Naag and Adi, 2010)]

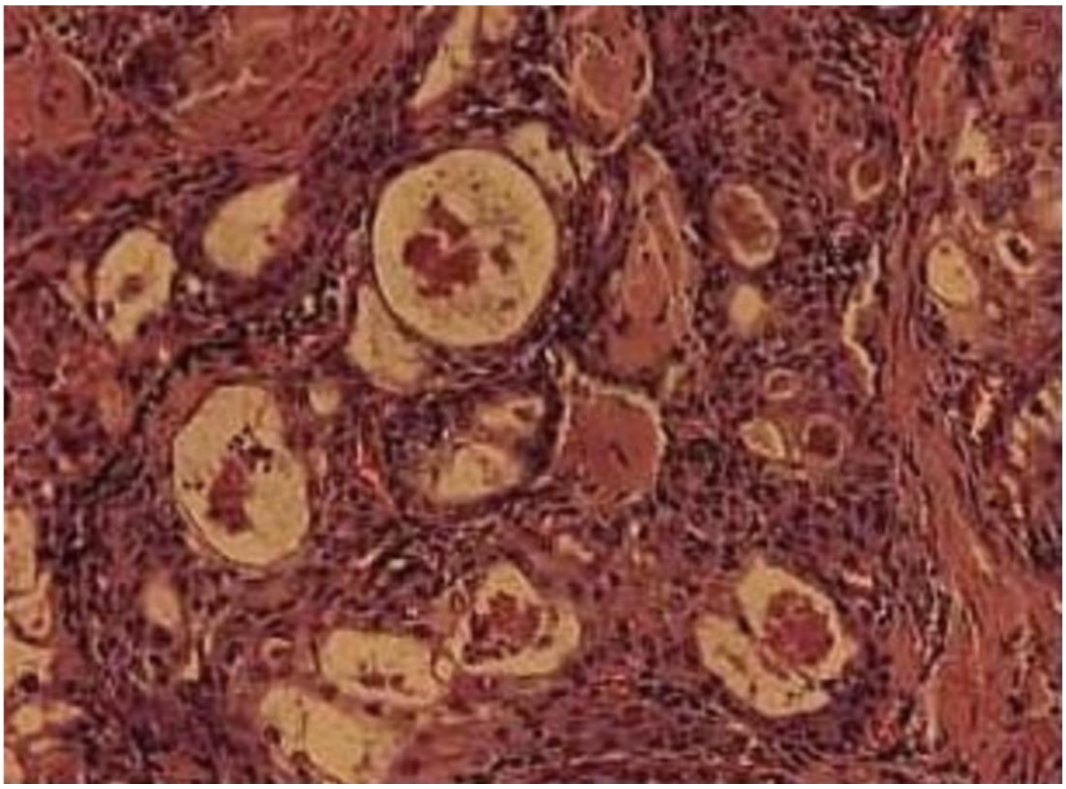

Fig.5 : Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in H&E (10X). [Courtesy: Article source (Naag and Adi, 2010)]

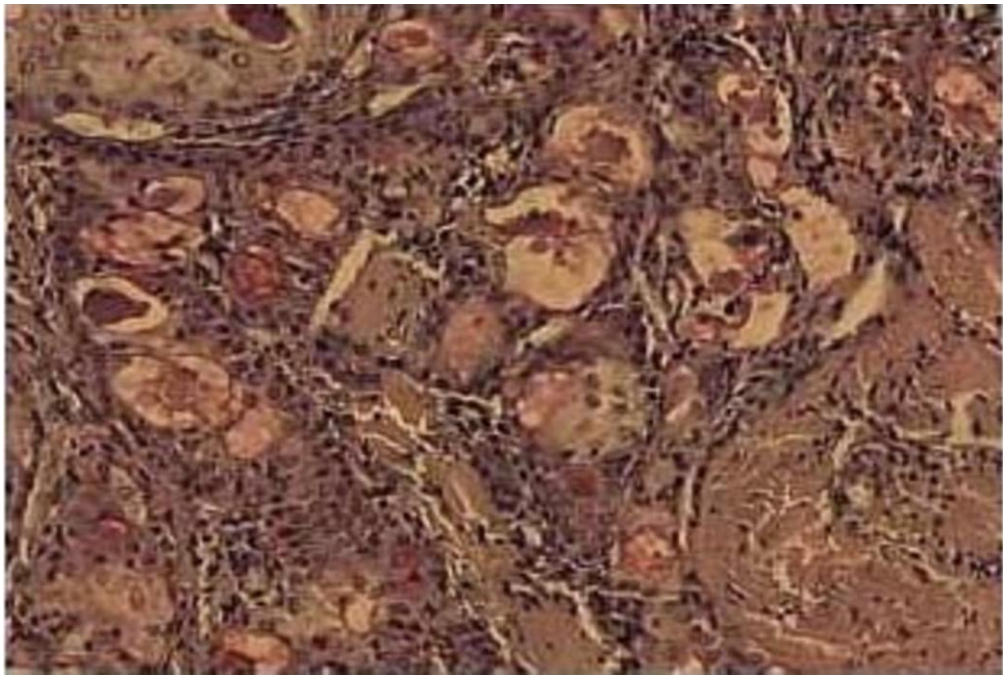

Fig.6 : Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in MC (10X). [Courtesy: Article source (Naag and Adi, 2010)]

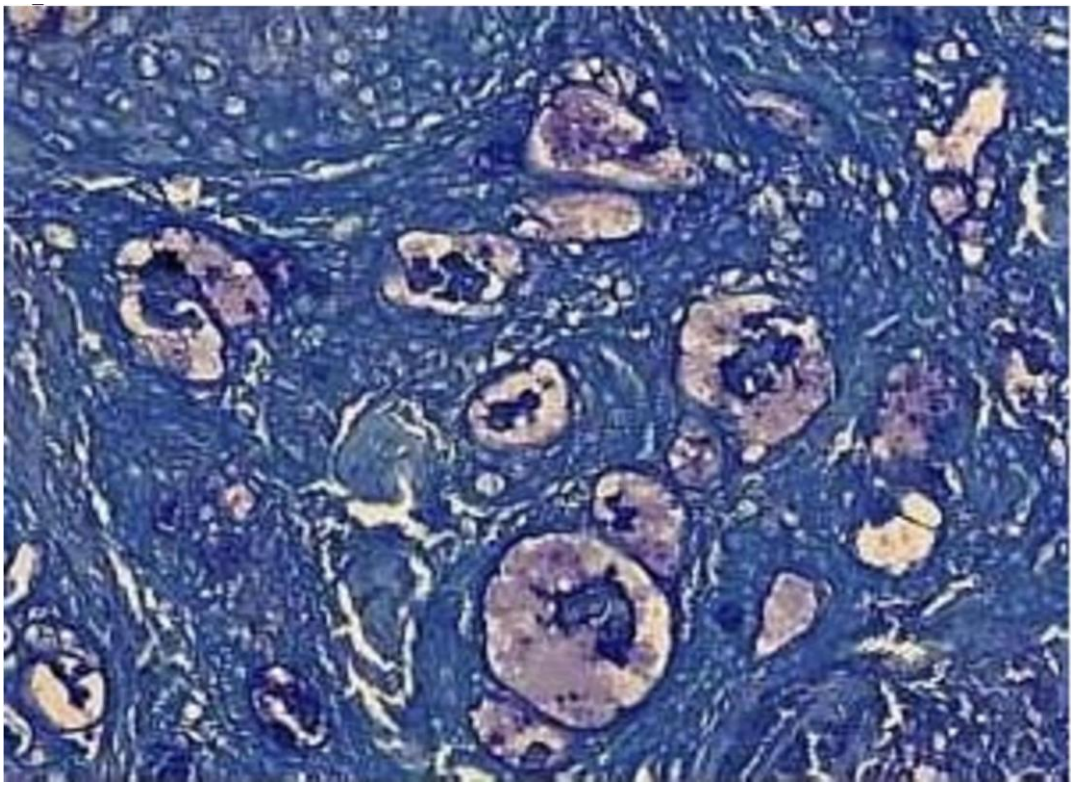

Fig.7 : Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in AF-AB (10X). [Courtesy: Article source (Naag and Adi, 2010)]

TABLES AND FIGURES

Figure- 1 : shows flow chart for study selection

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Figure- 2 : Photomicrograph showing ductal lumen and chondromyxoid areas of PA in H&E (10X) [Courtesy: Article source (Naag and Adi, 2010)]
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Figure- 7: Photomicrograph showing Mucous cells, Lumen, Epidermoid cells & stroma of MEC in AF-AB (10X) [Courtesy: Article source (Naag and Adi, 2010)]