Journal of Pharmaceutical Research International

33(57A): 323-329, 2021; Article no.JPRI.78091
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Oral Squamous Cells and Age Estimation in Exfoliative Cytology with Hematoxylin and Eosin Stain– A Quantitative Study

B. Keerthana [a], R. Priyadharshini [a*] and Palati Sinduja [a]

[a] Department of Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 77, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i57A34003

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/78091

Original Research Article

Received 06 October 2021
Accepted 12 December 2021
Published 14 December 2021

ABSTRACT

Introduction: Exfoliative cytology in age estimation is a simple, painless, less invasive collection of exfoliative cells from epithelial layers, used as a diagnostic aid for age estimation. The oral cavity is an ideal site for exfoliative epithelial cells with a physiological turnover of cells, turnover decreases as the age increases show age variation with cellular morphological changes. Age estimation is one of the important factors to identify an individual and also helps to know the chronological age of a person.
Aim: To analyze and estimate the age from buccal smear and comparing the average cellular size under Image morphometric analysis.
Materials and Methods: Buccal mucosal smears are taken using a wooden spatula in gentle motion of scraping and smeared on a clean glass slide and fixed in 95% ethanol immediately after smearing a minimum of around 15 minutes and stained with Haematoxylin and eosin stain. After staining, the cells were observed by microscope and measured by a paint tool. Pearson correlation analysis was done using SPSS software.
Results: The cell and nuclear size difference values observed using a Pearson correlation coefficient were statistically significant with p value<0.05 revealing that there is shrinkage in cells with increase in age.
Conclusion: Exfoliative cytology is a successful and vastly growing technology that is used for the detection of premalignant lesions.

**Corresponding author: E-mail: priyadharshinir.sdc@saveetha.com;*

Keywords: Age; buccal smear; cell size; estimation; exfoliative cytology; innovative technique; nuclear size.

1. INTRODUCTION

Exfoliative cytology in age estimation is a simple, painless, less invasive collection of exfoliative cells from epithelial layers, used as a diagnostic aid for age estimation [1]. Both quantitative and qualitative analyses of exfoliated cells are obtained with cell size, shape, Nuclear cytoplasmic ratio, nuclear density, and texture for diagnosis [2].

Age estimation is one of the important factors to identify an individual also helps to know the chronological age of persons in judicial proceedings, mass disasters, child marriage, elections, civil issues, refugees, dentistry, forensic, anthropology, and also in diseased conditions. The oral cavity is an ideal site for exfoliative epithelial cells with a physiological turnover of cells, turnover decreases as the age increases show age variation with cellular morphological changes [3]. Cytological smears are also used in Immunohistochemistry in tumor cell identification, RNA, DNA extract, and epigenetic alteration [4]. Identification of humans with sex and age helps in matching the missing humans through exfoliative cytology [5]. Variation in cytomorphology and nuclear morphology changes in oral epithelial cells are scraped and stained for cytological study and demonstration [6].

In the last few years, exfoliative cytology is the most common diagnostic methodology. Few reasons like errors in findings, less number of samples, and interobserver bias give false-negative results. For precise results with fewer false-negative results parameters like nuclear and cytoplasmic areas, the N: C ratio has to be evaluated correctly [7]. Johnston measures nuclear-cytoplasmic (N:C) ratios of normal and malignant epithelial cells [8]. Reagan underwent an elaborative study on the significance of normal and malignant cells. undertook a more extensive study [9]. Gold and stats analyzed factors like nuclear-cytoplasmic ratios from exfoliated cells of the oral cavity. [10]. Cowpe oral smears collected from diseased mucosa and contralateral areas of normal mucosa observed nuclear-cytoplasmic ratio are altered in the normal and diseased site [11].

Other methods like Radiovisiography for morphometric analysis of pulp-tooth ratio in lower canine [3]. Exfoliative cytology is based on microscopic examination of exfoliated epithelial cells after fixation and staining of the smears. Methods for collecting the samples are by Direct method by rubbing the mucosal surface, Indirect method is by aspiration with exfoliated cells and by Imprint method. The exfoliated cells are preserved in 10% methanol and stained [12]. Our team has extensive knowledge and research experience that has translate into high quality publications [13-32].

This study describes the quantitative analysis of age estimation with the oral smears where changes in nuclear size are seen with increasing age. Normal squamous cells can be collected from buccal mucosa, tongue, the floor of the mouth, gingiva, etc., This study was done quantitatively from the oral buccal smear and stained with Haematoxylin and eosin and examined for age-related changes in cells. The study aims to analyze and estimate the age from buccal smear and compare the average cellular size under Image morphometric analysis.

2. MATERIALS AND METHODS

The study was conducted in the department of pathology of Saveetha dental college with the ethical approval committee with ethical approval number IHEC/SDC/BDS/1955/01. The study was a simple, painless, and less invasive study and cost-efficient but included smaller populations. A sample size of 30 patients, divided into Group I as 10 individuals under the age group of 20-30 years of age, Group II 10 individuals above 60years of age, and GroupIII 10 individuals as control groups. Sample bias was stratified, validation of the procedure was done by a guide and expert pathologist. Smears are taken from the buccal mucosa from individuals of different age groups. Individuals with oral pigmentation, premalignant lesions, any other systemic illness, smoking, and alcohol habits are all excluded from the study.

Buccal mucosal smears are taken using a wooden spatula in gentle motion of scraping and smeared on a clean glass slide and fixed in 95% ethanol immediately after smearing a minimum of around 15 minutes and stained with Haematoxylin and eosin stain.

The stained smears image analysis was done by moving the slides in a zigzag manner from right

to left so that to avoid imaging the same cells again at 40X. Cell size has to be measured in both vertical and horizontal manner in image analysis software in micrometer (mm). Cells without overlapping are taken. 10 clear cells are taken from each slide and marked in a paint tool; manually projected images are captured in Olympus BX 41 Microscope. The cell and nuclear size difference values are observed using a Pearson correlation coefficient and were done using the software SPSS version 23. Dependent variables included age and independent variables included gender, height, weight.

3. RESULTS

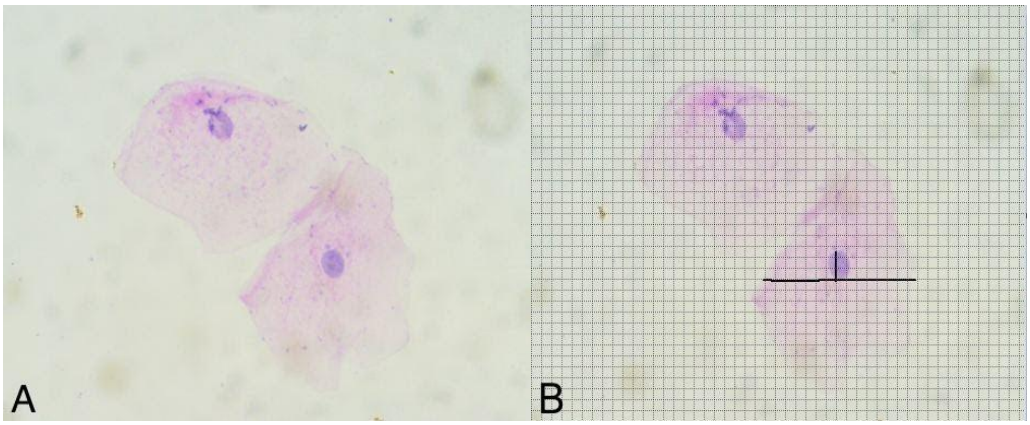

Fig. 1. (A) Represents the Haematoxylin and eosin-stained image (40x) of 30years old. (B) represents the measurement of a cell and nuclear diameter vertically and horizontally

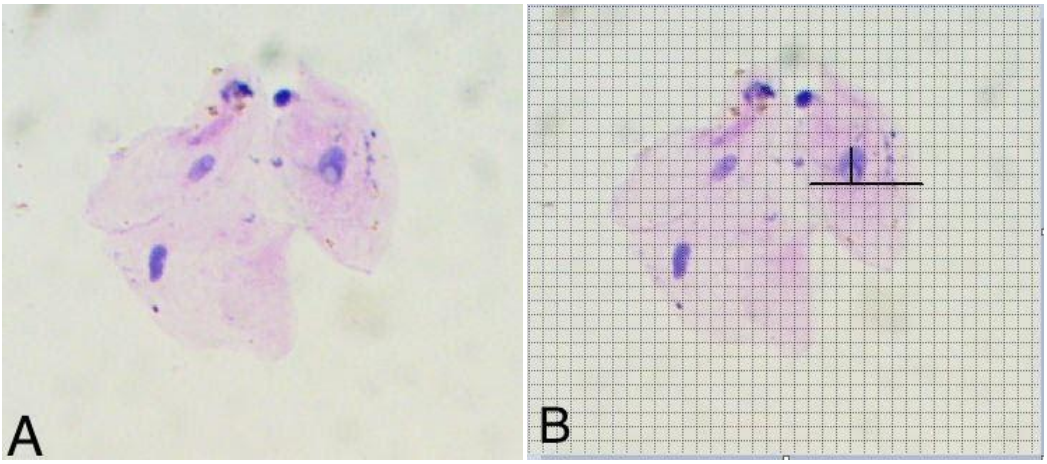

Fig. 2. (A) Represents the Haematoxylin and eosin-stained image (40x) of 60 years old. (B) represents the measurement of a cell and nuclear diameter vertically and horizontally.

Table 1. Cell diameter comparison between age groups showed Significant differences using the Pearson correlation coefficient

Group	Age(years)	Sample Size	Mean	Regression Coefficient
Group I	>30	20	53.77	0.889
Group II	<60	20	41.40	0.876

The Pearson correlation coefficient for variable cell size was found to be r = 0.8

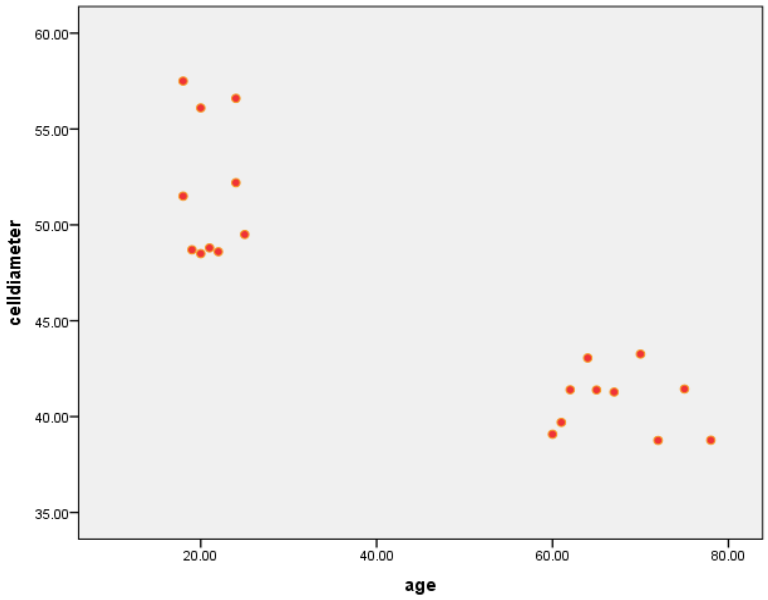

Fig. 3. Dotted graph depicting the relationship between age and cell diameter (n=20) with the decrease in cell diameter with an increase in age. The X-axis represents the age in years and the Y-axis represents cell diameter (µm)

Table 2. Nuclear diameter comparison between age groups showed Significant differences using a Pearson correlation coefficient

Group	Age (years)	Mean (µm)	Regression coefficient
Group I	>30	8.09	0.869
Group II	<60	5.79	0.899

The Pearson correlation coefficient for variable cell size was found to be r = 0.8

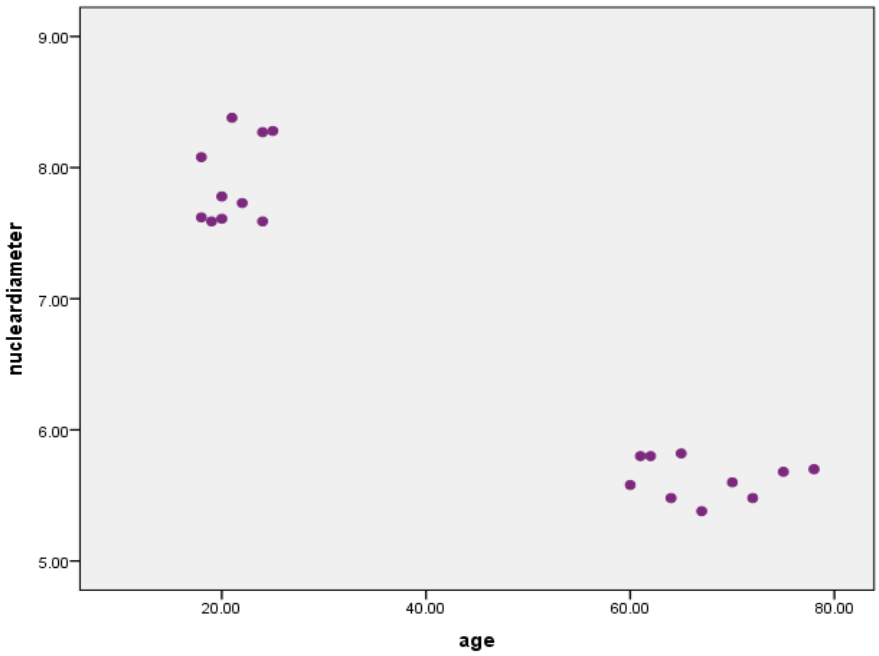

Fig. 4. Dotted graph depicting the relationship between age and nuclear diameter (n=20) with the decrease in nuclear diameter with an increase in age. The X-axis represents the age in years and the Y-axis represents the nuclear diameter (µm)

4. DISCUSSION

The age estimation of Pearson's correlation where the (Fig. 1) depicts Haematoxylin and eosin-stained image (40x) of 30 years old and (Fig. 2) depicts Haematoxylin and eosin-stained image (40x) of 60 years old. (Table 1) represents the cell diameter comparison between age groups showing a significant difference using the Pearson correlation coefficient. The average cell size varies from a minimum value of 40 µm/sq to a maximum value of 50 µm/sq**. (**Table 2) represents the nuclear diameter comparison between age groups showing a significant difference using the Pearson correlation coefficient. The cell size in group 1 ranged an overall average of 53.77 µm/sq. In group 2 the cell size varied ranging from an average cell size of 41.50 µm/sq. (Fig. 3) depicts a dotted bar graph showing the relationship between age and cell diameter (n=20) with the decrease in cell diameter with an increase in age. (Fig. 4) depicts a dotted bar graph showing the relationship between age and nuclear diameter (n=20) with the decrease in nuclear diameter with an increase in age. The results show that average cell size is diverse between different age groups. The Pearson correlation coefficient for variable cell size was found to be *r = 1* which was statistically significant showing that the cell size decreases with an increase in age. The distribution of cell size with various groups of different ages has significant differences, showing variation in cell size to be significant in different age groups.

Exfoliative cytology is a non-invasive method in collection of samples and in diagnosis [33]. Exfoliative cytology involves calculating cytomorphological changes in the cell with the nuclear-cytoplasmic ratio which comprises all the layers of the keratinized and non-keratinized layers of the epithelium. The staining is from pink to orange [2]. Stratum corneum is the compressed cell with condensed nuclear chromatin called Pyknosis followed by the disappearance of the nucleus with thin cornified cells [34]. Exfoliative cytology along with computer-based image analysis of cell size is an accurate, faster, accurate, and easier method. With increasing age variation of cell size irrespective of gender, determination reveals the repeated division of basal cells with the decreased renewal of cells followed by senescence of cells with increasing age also influenced by environmental factors with decreased epithelial turnover and cell organelles [35,36]. The morphology of normal basal cells is normal cell size with a larger nucleus around one-fourth of the cell size with cytoplasm which is basophilic. Prickle cell layer with cell size larger than stratum basale but smaller and intermediate nuclear size with flat and irregular cell size. Haematoxylin & eosin stain has been routinely used in regular histological examination of the tissues [37].

The limitations of the study included sample size, keratinized lesions show negative cytology, larger sampling size, and development in the technology of exfoliative cytology may overcome the limitation of the study. The future scope can be that exfoliative cytology is a promising diagnostic technology for premalignant or malignant lesions. It can be used as a diagnostic tool in the medical field.

5. CONCLUSION

Even though exfoliative cytology cannot take the place of biopsy for detecting the nature of lesions, it is vastly concerned in the estimation of oral lesions. Early detection of a premalignant oral lesion can help to increase the survival rate of patients suffering from pernicious conditions. Further studies with a larger study population will promise the role of oral exfoliative cytology.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was conducted in the department of pathology of Saveetha dental college with the ethical approval committee with ethical approval number IHEC/SDC/BDS/1955/01.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shetty DC, Wadhwan V, Khanna KS, Jain A, Gupta A. Exfoliative cytology: A possible tool in age estimation in forensic odontology. J Forensic Dent Sci. 2015 Jan;7(1):63–6.

2. Hande AH, Chaudhary MS. Cytomorphometric analysis of buccal mucosa of tobacco chewers. Rom J Morphol Embryol. 2010;51(3):527–32.
3. Nallamala S, Guttikonda VR, Manchikatla PK, Taneeru S. Age estimation using exfoliative cytology and radiovisiography: A comparative study. J Forensic Dent Sci. 2017 Sep;9(3):144–8.
4. Ilayaraja V, Priyadharshini TK, Ganapathy N, Yamunadevi A, Dineshshankar J, Maheswaran T. Exfoliative cytology for age estimation: A correlative study in different age groups. Journal of Indian Academy of Dental Specialist Researchers. 2018;5(1).
5. Kumaresan GD, Jagannathan N. Exfoliative cytology—A predictive diagnostic tool. Int J Pharm Pharm Sci. 2014;6(5):1–3.
6. Chaudhary R, Sahni P, Shylaja MD, Patel A. Age estimation by exfoliative cytology: New era of noninvasive forensic science. International Journal of Forensic Odontology. 2018 Jan 1;3(1):40.
7. Patel PV, Kumar S, Kumar V. Quantitative cytomorphometric analysis of exfoliated normal gingival cells. Journal of Cytology/Indian. 2011;2(3):171–5.
8. Johnston DG. Cytoplasmic: Nuclear ratios in the cytological diagnosis of cancer. Cancer. 1952 Sep;5(5):945–9.
9. Reagan JW, Hamonic MJ, Wentz WB. Analytical study of the cells in cervical squamous-cell cancer. Lab Invest. 1957 May;6(3):241–50.
10. Goldsby JW, Staats OJ. Nuclear changes of intraoral exfoliated cells of six patients with sickle-cell disease [Internet]. Oral Surgery, Oral Medicine, Oral Pathology. 1963;16:1042–8. Available:http://dx.doi.org/10.1016/0030-4220(63)90216-0
11. Ogden GR, Cowpe JG, Green MW. Effect of radiotherapy on oral mucosa assessed by quantitative exfoliative cytology [Internet]. Journal of Clinical Pathology. 1989;42:940–3.
12. Available:http://dx.doi.org/10.1136/jcp.42.9.940
13. Warnakulasuriya S, Greenspan JS. Textbook of oral cancer: Prevention, diagnosis and management. Springer Nature. 2020;452.
14. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. Am J Cancer Res. 2020 Aug 1;10(8):2546–54.
15. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. Hypertens Res. 2020 Dec;43(12):1459–61.
16. Sivakumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in Streptococcus sp., using computational approach. Journal of King Saud University - Science. 2020 Jan 1;32(1):904–9.
17. Smiline Girija AS. Delineating the immuno-dominant antigenic vaccine peptides against gacS-sensor kinase in *Acinetobacter baumannii*: An in silico investigational approach. Front Microbiol. 2020 Sep 8;11:2078.
18. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. Journal of King Saud University - Science. 2020 Dec 1;32(8):3380–7.
19. Girija ASS. Fox3^{+} CD25^{+} CD4^{+} T-regulatory cells may transform the nCoV's final destiny to CNS! J Med Virol [Internet]; 2020 Sep 3. Available:http://dx.doi.org/10.1002/jmv.26482
20. Jayaseelan VP, Ramesh A, Arumugam P. Breast cancer and DDT: Putative interactions, associated gene alterations, and molecular pathways. Environ Sci Pollut Res Int. 2021 Jun;28(21):27162–73.
21. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol. 2021 Feb;122:105030.
22. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. Pharmaceutical-Sciences [Internet]. 2020;82(2).
23. Available:https://www.ijpsonline.com/articles/targeting-nm23h1mediated-inhibition-of-tumour-metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html

24. Girija SA, Priyadharsini JV, Paramasivam A. Prevalence of carbapenem-hydrolyzing OXA-type β-lactamases among *Acinetobacter baumannii* in patients with severe urinary tract infection. Acta Microbiol Immunol Hung. 2019 Dec 9;67(1):49–55.
25. Priyadharsini JV, Paramasivam A. RNA editors: Key regulators of viral response in cancer patients. Epigenomics. 2021 Feb;13(3):165–7.
26. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach. Acta Virol. 2020;64(1):93–9.
27. Girija As S, Priyadharsini JV, Paramasivam A. Prevalence of Acb and non-Acb complex in elderly population with urinary tract infection (UTI). Acta Clin Belg. 2021 Apr;76(2):106–12.
28. Arvind P TR, Jain RK. Skeletally anchored forsus fatigue resistant device for correction of Class II malocclusions-A systematic review and meta-analysis. Orthod Craniofac Res. 2021 Feb;24(1):52–61.
29. Venugopal A, Vaid N, Bowman SJ. Outstanding, yet redundant? After all, you may be another Choluteca Bridge! Semin Orthod. 2021 Mar 1;27(1): 53–6.
30. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. Clin Oral Investig. 2019 Sep;23(9):3543–50.
31. Varghese SS, Ramesh A, Veeraiyan DN. Blended module-based teaching in biostatistics and research methodology: A retrospective study with postgraduate dental students. J Dent Educ. 2019 Apr;83(4):445–50.
32. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of *Streptococcus mutans*, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial [Internet]. Clinical Oral Investigations. 2020;24:3275–80.
Available:http://dx.doi.org/10.1007/s00784-020-03204-9
33. Arun Kishore RN, Priyadharshini R, Palati Sinduj. f6cacy of Methylene Blue and Papanicolaou Stain in sex determination-A comparative study. J Res Med Dent Sci. 2021;9(10):109-115.
34. Anuradha A, Sivapathasundharam B. Image analysis of normal exfoliated gingival cells. Indian Journal of Dental Research. 2007;18(2):63.
35. Sakuma A, Ohtani S, Saitoh H, Iwase H. Comparative analysis of aspartic acid racemization methods using whole-tooth and dentin samples. Forensic Sci Int. 2012 Nov 30;223(1-3):198–201.
36. Mehrotra R. Oral cytology: A concise guide. Springer Science & Business Media. 2012;176.
37. Sri ED, Priyadharshini R, Sinduja P, Meghashree V. Comparison of efficacy of the Pap and H & E in identifying the various changes in liver samples of high fat diet induced rat - In vitro study. Journal of Pharmaceutical Research International. 2021;33(54A):364-380.
DOI: 10.9734/jpri/2021/v33i54A33757

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/78091