ORIGINAL RESEARCH

Comparison of PAP and Giemsa stain for determination of Barr bodies in transgender patients

Sindhuja A S, Revathi.U, Sai Lakshmi L.J., Nadeem Jeddy

Abstract

Introduction: Barr bodies are inactivated X chromosomes that serve as a visual marker for chromosomal sex differences. They play a key role in genetic sex determination, as their presence or absence helps to assess an individual's sex. It is crucial in cases involving disfigured, decomposed bodies, medicolegal investigations, sports, & civil rights matters. The present study aims to compare the effectiveness of PAP and Giemsa stains in identifying Barr body in transgender patients.

Materials and Methods: A total of 45 individuals comprising of 15 (Male, female & transgender) were considered for the study. Buccal smears were obtained from each patient, stained with PAP & Giemsa stains and studied for identification of Barr bodies. Statistical significance was evaluated using the Kruskal-Wallis or one-way ANOVA tests (among 3 groups) Independent sample t-test or Mann-Whitney test (between 2 stains).

Results: Examination of PAP and Giemsa stained slides showed absence of Barr body in males; 100% and 80% in female; 6.67% in transgenders respectively. PAP staining had a sensitivity of 100%, specificity of 98%, and accuracy of 97%, while Giemsa staining had a sensitivity of 100%, specificity of 92.3%, and an accuracy of 95.83%.

Conclusion: Nuclear sexing using Barr bodies is one of the reliable method for gender determination. Both PAP and Giemsa stains are effective for Barr body identification, with PAP and Giemsa stain showing accuracy of 97% and 96%, making PAP stain more efficient in identification of Barr bodies.

Key Words: Barr bodies, Giemsa stain, Oral exfoliative cytology, PAP stain, transgender.

Introduction

Forensic odontology is the study and application of dental science in legal investigations, and it plays a key role in identifying individuals in cases of disaster, mass casualty events, or criminal investigations. In forensic odontology, Barr bodies can be used as a tool for gender determination, especially in cases where the biological sex of an individual needs to be established from dental or other tissue samples[1]. For Barr body identification buccal cells can be used, which can be easily obtained from a person by non-invasive sample collection methods that can be preserved for later analysis. In cases where other tissue samples are not available or have deteriorated, buccal mucosal cells offer a practical alternative. Other methods for gender determination, like DNA analysis, are more accurate, but Barr body detection can still be a useful initial screening tool, especially in scenarios where resources or time are limited.

Barr bodies representing the inactivated X chromosomes in somatic cells serve as a key cytogenetic marker for determining chromosomal sex. The presence or absence of Barr bodies can be used to assess chromosomal sex, which may be of particular interest in transgender individuals undergoing hormone replacement therapy (HRT) or gender-affirming surgeries[2]. Within human identification gender determination plays a very important role in identification of the par-

Department and Institution Affiliation: Post Graduate, Department Of Oral Pathology and Microbiology, Dr. M.G.R. Educational And Research Institute, University in Chennai, Tamil Nadu. Thai Moogambigai Dental College And Hospital, Chennai, India

Corresponding author: Dr. Sindhuja A S, No. 59 kummalamman koil street, Tondiarpet, Chennai- 600081 Email Id: saigeethapriya@gmail.com

How to cite the article: Sindhuja A S, Revathi.U, Sai Lakshmi L.J., Jeddy N. Comparison of PAP and Giemsa stain for determination of Barr bodies in transgender patients.. J. Oral Maxillofac Pathol J 2025; 16(2); 196-201.

Source of Support: Nil

Conflict of Interest: None

ticular victim[3]. Some of the nuclear sexing methods include karyotyping, examination of Barr bodies, Fluorescent body study[4].

Barr bodies are Feulgen positive, heteropyknotic and basophilic intranuclear structures which are seen during the interphase of cell cycle[5]. These (named after Murray Barr) are inert X chromosome that are always fewer than the total number of X chromosome[6] which are stainable structures that are approximately 1 micrometer in diameter and are flat or triangular[7]. It is commonly located on the side of the nuclear membrane and can be seen on the periphery of the nuclear membrane[8]. The study of Barr body is advantageous because it can be studied under ordinary compound light microscopy. A variety of staining methods such as PAP, orcein, crystal violet and fluorescent staining have been used to identify Barr bodies[9]. However, comparative analyses of these stains in transgender individuals remain limited. This study uniquely evaluates and compares the effectiveness of Papanicolaou (PAP) and Giemsa stains specifically in the transgender population. Given the notable lack of cytogenetic studies involving transgender individuals, this research

contributes to the growing body of inclusive scientific data and aids in establishing reference standards for chromatin-based studies.

Therefore, the present study aims to determine the presence of Barr bodies in exfoliated buccal cells in male, female, and transgender individuals and also to compare the efficacy of PAP and Giemsa stain under light microscopy.

Materials and methods

The study was conducted in the outpatient department after obtaining approval and ethical clearance from Institutional Review Board (IRB Approval Number: 236/2024/IEC/TMDCH). A total of 45 healthy patients were recruited in the study. Male and female patients were recruited from the outpatient department. Transgender patients were recruited from Transgender Rights Association Kolattur. The study was conducted over a period of 3 months. A written consent was obtained from all participants.

SAMPLE SIZE:

The sample size was estimated based on previous cytological studies comparing staining techniques for Barr body detection, as well as considering the practical limitations and availability of transgender participants. Since there is a paucity of literature specifically involving transgender individuals, a pilot design was adopted.

Group I consist of 15 male patients, Group II consist of 15 female patients, and Group III consist of 15 transgender patients.

INCLUSION AND EXCLUSION CRITERIA:

All participants were healthy individuals above the age of 18 years. To maintain consistency, individuals with systemic diseases or harmful oral habits were excluded from the study. For patients in Group III, there were no medical records available documenting their chromosomal analysis or gender investigation. Therefore, their self-identified gender was considered the definitive criterion for inclusion in Group III.

Among the 15 transgender patients, 14 were male-to-female (MTF) based on self-identification and expressed their gender identity through feminine dress and behaviour. Of these, five had undergone sex reassignment surgery (SRS), while the rest were either in the pre-operative stage or had chosen not to undergo surgical transition. Additionally, all 14 MTF individuals had received hormonal therapy to enhance feminine characteristics. One patient was female-to-male (FTM), also self-identified, and presented as male through masculine dress and behaviour.

Sample collection

In the present study prior to sample collection, participants were instructed to rinse their mouth with water. The buccal mucosa was then gently but firmly scraped using a wooden spatula to collect buccal cells. The collected sample was transferred onto two clean glass slides, creating two smears per individual. The smears were rapidly fixed in 95% ethyl alcohol. After staining with PAP and Giemsa, the slides were mounted with a cover slip and examined under a 40x light microscope.

Table 1: Percentage of Barr Bodies among three groups with respect to PAP and Giemsa

		Barr bodies (PAP Stain)				Barr bodies (Giemsa stain)			
Study groups	No. of patients	Present	%	absent	%	Present	%	absent	%
Group I (Male)	15	0	0	15	100	0	0	15	100
Group II (Female)	15	15	100	0	0	12	80	3	20
Group III (Transgender)	15	1	6.67	14	93.33	1	6.67	14	93.33
Total	45	16	35.55	29	64.44	13	28.89	32	71.11

(Percentage - %)

Papanicolaou (PAP) Stain Procedure:

Smears were immediately fixed in 95% alcohol for 5–15 minutes, followed by sequential hydration through decreasing ethanol concentrations (80%, 70%, 50%). After rinsing in distilled water, slides were stained with Harris's hematoxylin for 2–3 minutes, differentiated in 0.25% and 0.5% HCl, and blued under tap water. After nuclear staining assessment, slides were dehydrated through ascending alcohol concentrations. Cytoplasmic staining was performed with Orange G-6 and EA 36/EA-50, with intermediate alcohol rinses. Final dehydration was done with absolute alcohol, cleared in xylene, and mounted with a neutral medium.

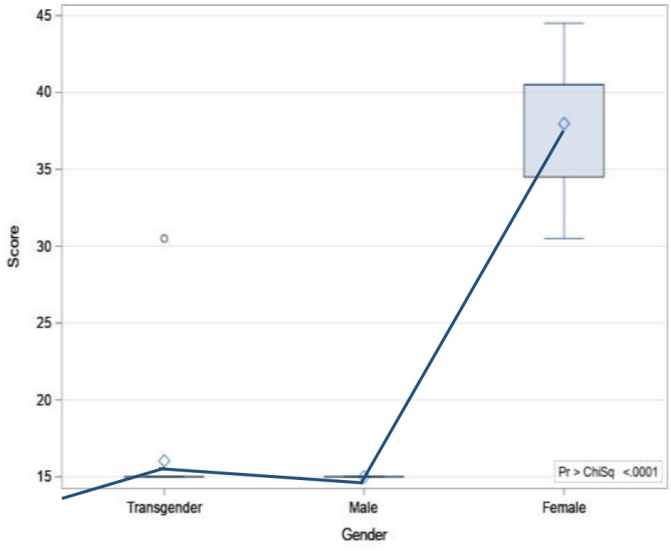
Giemsa Staining Procedure:

Air-dried smears were fixed in methanol for 10 minutes and allowed to air-dry completely. Slides were stained with 5% Giemsa solution (prepared in tap water) for 20 minutes, followed by gentle washing in tap water to remove excess stain.

Table 2: Comparison of mean barr body positive cells between groups with respect to PAP and Giemsa

	Group			
	Female	Male	Trans-gender	P
Positive cells – PAP Stain				<0.001*
n	15	15	15	
Mean	2.7	0	0.1	
Std. Dev	1.1	0	0.3	
1st quartile	2.0	0	0	
Median	3.0	0	0	
3rd quartile	3.0	0	0	
Positive cells – Gemisa Stain				<0.001*
n	15	15	15	
Mean	1.0	0	0.1	
Std. Dev	0.8	0	0.3	
1st quartile	0	0	0	
Median	1.0	0	0	
3rd quartile	2.0	0	0	

*P<0.05 - Statistically Significant



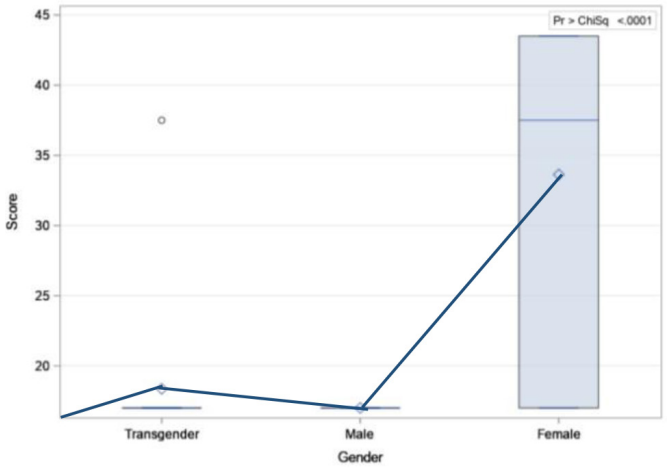
Graph -1 Distribution of Wilcoxon scores for no. Of cells in PAP stain per 50

Table 3: Sensitivity and specificity of PAP and Giemsa stain for Sex determination

	PAP Stain	Giemsa Stain
Sensitivity	100%	100%
Specificity	98%	92.3%
Accuracy	97%	95.83%

Table 4: Difference between PAP Stain and Giemsa stain used in the study

PAP	Giemsa
Time consuming	Time saving
Screening of more samples are made easy	Screening of More samples are difficult
Expensive	Cost efficient
There is less chance of stained slides getting faded.	There is increased chance of stained slides getting faded
Accuracy and efficacy on staining is comparatively more	Accuracy and efficacy on staining is comparatively less
Nuclear details are interpreted more efficiently	Cytoplasmic details are interpreted more efficiently



Graph -2 Distribution of Wilcoxon scores for no. Of cells in Giemsa stain per 50

Screening of slides:

Slides that had been stained and mounted were systematically examined. 50 cells per slides were counted. During screening, a structure was considered a Barr body if it appeared either freely within the nucleoplasm or along the nuclear membrane, and displayed a circular, disc-like, or triangular shape. Two observers examined the slides independently.

Statistical analysis:

Statistical analysis was done using IBM SPSS Software (version 29.0.2) and it's significance was assessed using one way ANOVA test and Kruskall Wallis test(test for 3 group comparison). For the comparison of two independent samples, Mann Whitney test was used. Kappa statistics was used to find the agreement between the observers in identifying barr bodies.

Results

The patients participated in the study were above 18 years of age. When PAP stained slides were examined, the percentage of barr bodies in males was 0%, in females it was 100% and in Transgender it was 6.67%. In Giemsa stained smears, the percentage of barr bodies in males it was 0%, in females it was 80% and in transgenders it was 6.67% (TABLE1). There was a significant difference ($P< 0.01$) in mean barr body positive cells when compared between the 3 groups with respect to both PAP and Giemsa stain (TABLE2). The sensitivity and specificity of PAP for sex determination was 100% and 98% with an accuracy of 97 % whereas, for Giemsa stain the sensitivity was 100% and specificity was 92.3 % with an accuracy of 95.83%(TABLE 3). Kappa coefficient indicates (0.75) a moder-

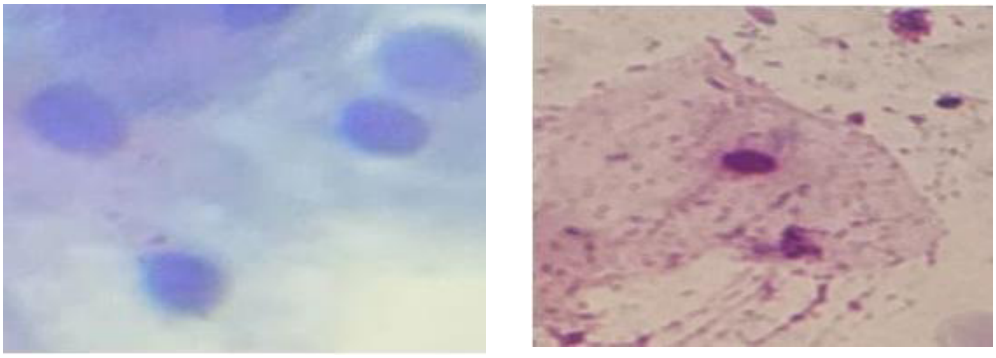


Fig. 1 (a) PAP stain and **1(b)** Giemsa stain in male patients.

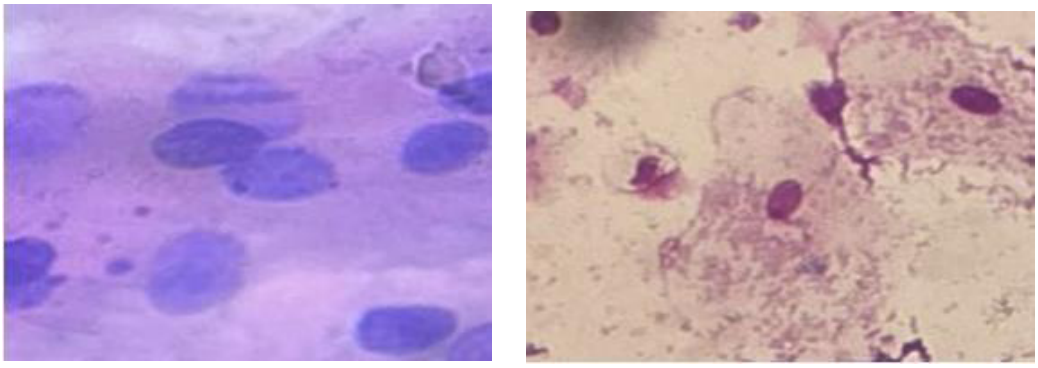


Fig. 2(a) PAP stain and **2(b)** Giemsa stain in female patients.

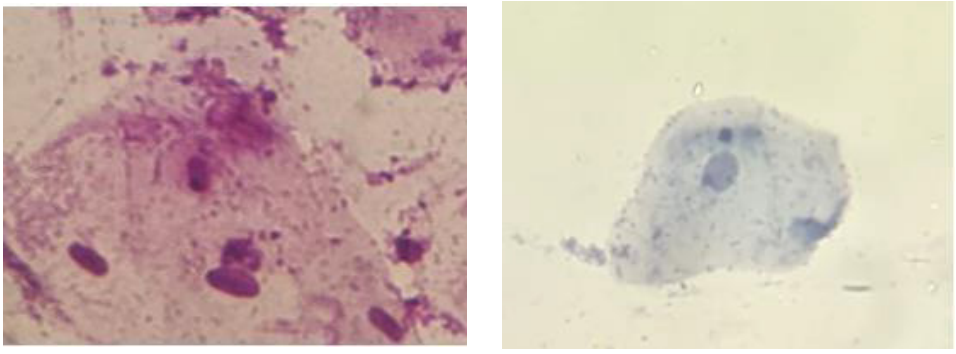


Fig. 3 (a) PAP stain and **3(b)** Giemsa stain in transgender patients.

ate agreement between the observers (PAP and Giemsa stain) in identifying Barr bodies.

Discussion

Sex determination is a fundamental aspect of personal identification in forensic and clinical settings. One of the reliable indicators of biological sex is the presence of the Barr body, which represents the inactivated X chromosome in somatic cells of genetic females. This process of X chromosome inactivation, known as lyonization, ensures dosage compensation between males (XY) and females (XX)[10]. The Barr body is typically located either at the periphery of the nuclear membrane or free within the nucleoplasm, and it assumes a characteristic circular, disc-shaped, or triangular morphology.

Various studies have investigated the presence of sex chromatin in different human tissues such as buccal epithelial cells, pulp fibroblasts, cervical smears, skin, and hair follicles11. Females, having two X chromosomes, are generally chromatin-positive due to the presence of a Barr body, whereas males, with a single X chromosome, are chromatin-negative[12].

In the present study, Barr bodies were observed in 100% of female participants using the Papanicolaou (PAP) stain, while the Giemsa stain revealed Barr bodies in only 80% of females. Among transgender individuals, 6.6% showed positive staining for Barr bodies with both methods, possibly reflecting chromosomal mosaicism or hormonal influences, while none of the male participants exhibited Barr body positivity. These findings emphasize the higher sensitivity of PAP staining in identifying Barr bodies compared to Giemsa.

Our findings corroborate the results reported by Hermann W et al., who studied 100 subjects and found that all genetically female participants were Barr body-positive using PAP staining, with percentages ranging between 10% and 32%, whereas all male participants were negative13. Similarly, Arun Kishore RN et al. compared PAP and methylene blue stains and found that PAP stain had a sensitivity of 97% and specificity of 98%, outperforming methylene blue in both metrics. Their reported accuracy for PAP stain was 97%, indicating its superior diagnostic value[14,15].

Furthermore, our results are in alignment with the study conducted by Aishwarya Lakshmi et al., who examined exfoliated buccal epithelial cells in 90 individuals (30 each of males, females, and transgender participants) using PAP and Acridine Orange stains. They reported Barr body positivity in all females (3%–5%), complete negativity in males, and 1%–3% positivity in transgender participants, highlighting the variable chromatin pattern in transgender individuals, possibly influenced by gender-affirming therapies or chromosomal diversity[16].

Overall, the study reinforces that PAP stain is more effective than Giemsa for Barr body detection, particularly in ambiguous or non-binary cases, and highlights the importance of inclusive cytogenetic evaluation in contemporary practice.

Conclusion

The present study reaffirms that nuclear sexing through Barr body identification is a simple and effective method for determining chromosomal sex. In transgender individuals, the presence or absence of Barr bodies corresponds to chromosomal sex rather than gender identity, underscoring the importance of distinguishing between biological and gender markers in clinical and research settings. This study helps fill the existing gap in cytogenetic data for the transgender population, offering valuable baseline insights. Among the staining techniques evaluated, both Papanicolaou (PAP) and Giemsa stains proved effective; however, PAP demonstrated superior accuracy (97%), establishing it as the more reliable method for sex chromatin analysis.

Acknowledgments

We gratefully acknowledge the invaluable contribution of Dr. Suruthi Sundaramurugan, whose expertise in statistical analysis greatly enhanced the integrity and depth of this study. Their guidance in designing the analytical framework, interpreting complex data sets, and ensuring the robustness of the results was instrumental throughout the research process.

We would also like to extend our heartfelt appreciation to Mrs. Machagandhi S.M, the pathology laboratory technician, for their meticulous work and technical support throughout this study.

References

1. Nagare SP, Chudhari RS, Birangane RS, Parkarwar PC. Sex determination in forensic identification, a review. J forensic dental science 2018; 10:61-6
2. Mohan G, Dharman S. Sex determination and personal identification using frontal sinus and nasal septum – A forensic radiographic study. Indian Forensic Med Toxicol 2019; 13: 125
3. Nagamori H. Sex determination from plucked human hairs without epithelial root sheath. Depigmentation of melanin in the hair cortex before Feulgen reaction. Forensic Sci Inter 1980; 15: 169 – 175
4. Preethikaa S, Brundha MP. Awareness of diabetes mellitus among general population. Res J Pharm Technol 2018; 11: 1825-1829
5. Nagamori H, Ohno Y, Uchima E, et al. Sex determination from buccal mucosa and hair root by the combined treatment of quinacrine staining and fluorescent Feulgen reaction using a single specimen. Forensic Sci Int 1986; 31: 119-128
6. Nivedhita G, Brundha MP. Analysis of Papanicolaou stain on peripheral smear compared tomLrishman's stain: A prospective study. Int J clinico patholncorrel 2020; 4;40 7.
7. Datar U, Angadi PV, Hallikerimath S, et al. Cytological assessment of Barr bodies using aceto-orecin and papanicolaou stains in buccal mucosal smears and their sex estimation efficacy in an Indian sample. Acta Cytological 2013; 57: 516-521
8. Timothy CN, Samyuktha PS, Brundha MP. Dental pulp stem cells in regenerative medicine—A literature review. Res J Pharm Technol2019; 12: 4052-4056
9. Efficacy of methylene blue and Papanicolaou stain in sex determination – a comparative study Arun Kishore RN, Priyadarshini R, Palati Sinduj
10. Lyon MF. Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature. 1961;190:372–373.
11. Narayana BL, Prakash BS. Sex chromatin and its application in forensic science. J Forensic Med Toxicol. 2008;25(1):25–30.
12. Prakash J, Ramesh S, Chaitanya B. Barr body: A cytogenetic marker for gender identification. Indian J Med Sci. 2012;66(4):101–104.
13. Hermann W, Sobol M. Detection of Barr bodies in exfoliated oral mucosa cells using PAP staining. Acta Cytol. 2003;47(3):455–458.
14. Arun Kishore RN, Swathi K, Shilpa S. Comparative evaluation of PAP and methylene blue stains in identification of sex

chromatin in buccal smears. J Clin Diagn Res. 2014;8(10):ZC34–ZC36.

15. Swathi K, Arun Kishore RN. Sex determination using exfoliative cytology: A study of Barr bodies in buccal smears using different stains. Int J Forensic Odontol. 2013;1(1):15– 18
16. Lakshmi A, James A, Annasamy R, Krishnan R. Determination of Barr bodies in Transgender Patients in India - A comparative study. J Oral Maxillofac Pathol. 2023 OctDec;27(4):674-678. doi: 10.4103/jomfp.jomfp_342_23. Epub 2023 Dec 20. PMID: 38304528; PMCID: PMC10829437.
17. Rimoin DL, Pyertiz RE, Korf B, editors. Emery and Rimoin's essential medical genetics. Elsevier; 2013.
18. Ramakrishnan K, Sharma S, Sreeja C, Pratima DB, Aesha I, Vijayabanu B. Sex determination in forensic odontology: A review. J Pharm Bioallied Sci. 2015 Aug;7(Suppl 2):S398-402. doi: 10.4103/0975-7406.163469. PMID: 26538886; PMCID: PMC4606628.
19. Saxena S, Sharma P, Gupta N. Experimental studies of forensic odontology to aid in the identification process. J Forensic Dent Sci. 2010 Jul;2(2):69-76. doi: 10.4103/09751475.81285. PMID: 21731343; PMCID: PMC3125956.
20. Chowdhury RM, Singhvi A, Bagul N, Bhatia S, Singh G, Goswami S. Sex determination by amplification of amelogenin gene from dental pulp tissue by polymerase Chain Reaction. Indian J Dent Res. 2018 Jul-Aug;29(4):470-476. doi: 10.4103/ijdr.IJDR_274_17. PMID: 30127199.
21. Baby TK, Thomas P, Palani J, Pillai RK, Ramakrishnan BP. Sex determination efficacy of Papanicolaou and acriflavine Schiff stains in buccal smears. J Forensic Dent Sci. 2017 Jan Apr;9(1):46. doi: 10.4103/0975-1475.206480. PMID: 28584481; PMCID: PMC5450489.
22. Mittal T, Saralaya KM, Kuruvilla A, Achary C. Sex determination from buccal mucosa scrapes. Int J Legal Med. 2009 Sep;123(5):437-40. doi: 10.1007/s00414-008-0285-8. Epub 2008 Sep 24. PMID: 18813940.
23. Anoop UR, Ramesh V, Balamurali PD, Nirima O, Premalatha B, Karthikshree VP. Role of Barr bodies obtained from oral smears in the determination of sex. Indian J Dent Res. 2004 Jan-Mar;15(1):5-7. PMID: 15682788.