Sex Estimation Efficacy of Saffranine and Methylene Blue in Cytological Assessment ofBarr bodies: A Comparative Study.

Vataparthi Pravallika[1,2], Manisai Koduri[3], Sony Gunaganti[3], Lakshmi Panicker[4]

[1]Department of Forensic Science, School of Paramedics and Allied Health Sciences, Centurion University of Technology and Management, Andhra Pradesh, India.
[2]Department of Forensic Science, PIAS, Parul University, Vadodara, Gujarat, India.
[3]Department of Optometry, School of Paramedics and Allied Health Sciences, CenturionUniversity of Technology and Management, Andhra Pradesh, India.
[4]Department of Forensic Science, School of Sciences, Jain (Deemed-to-be-University)

ABSTRACT:

Purpose:

Testing Barr bodies presence has a huge application in multidisciplinary areas of research and practice. Barr bodies help predict cause of infertility, chance of tumors, cancer, efficiency in findings transplanted Retinal pigment epithelium and much more. In most criminal investigations and forensic analysis, establishing the identity of gender is crucial for progressive investigation and avoiding tampering with evidence. Gender identification can also be performed using basic staining procedures. The current study aimed to see efficacy of sex determination using cytological assessment of Barr bodies with Saffranine for the first time.

Methods: A total of 60 Buccal samples (30 females) from healthy individuals were collected. Each sample is built into two smears (n=120). One set of smears stained with Saffranine and another with Methylene blue. Stained smears are visualized under 40X mag using binocular compound microscope immediately and graded sample on the basis of details seen.

Results:

Staining scores by Saffranine is higher among both the genders. Mann-Whitney U test has concluded that Saffranine has a better staining performance with (U = 31.0) (Overall Md =5) and P < 0.001 than Methylene Blue stain among females with (U = 51.0) (Overall Md =3) and P < 0.001. Correspondingly, the sensitivity and specificity for sex estimation using Safranine are 100% and 93.3%, respectively, whereas sensitivity and specificity using Methylene blue are 93.3% and 93.3%.

Overall, it is conspicuous that Saffranine has superior staining performance and, thereby, sex estimation efficacy than Methylene blue.

Conclusion:

Barr body visualization have a significant application in multidisciplinary research domains. Basic staining procedures are reliable, fast, accurate and cost effective in determining the sex of an individual. Overall, Saffranine stain have higher efficacy compared to Methylene blue in sex determination.

*e*ISSN1303-5150

DOINumber:10.14704/nq.2022.20.8.NQ44629 **NeuroQuantology2022;20(8):6019-6032**

Background

Testing Barr bodies presence has a huge application in multidisciplinary areas of research and practice. In females, X chromosomes become inactivated and form Barr bodies. This process of inactivation is known as Lyonization. Barr bodies are seen as spherical, triangular, plano-convex, biconvex, and seen at the periphery of the nucleus in every somatic cell. The size of Barr bodies bearing 0.8 X 1.1 microns can be seen easily using a microscope [1]. In an attempt to morphologically distinguish male and female neurons and the behaviour of nucleolar satellites, Murray L Bar and Ewart G Bertam have identified Barr Bodies as a nucleolar satellite in male and female cat neurons. According to them, 30-40% of female cells bear Barr bodies and travel away from the nucleus during intense ribosome nucleoprotein synthesis [2].

Barr bodies help predict the cause of infertility, the chance of tumours, and much more. Barr bodies can be obtained from buccal smears, hair follicles, and odonatological samples and are crucial tools in forensic research and investigation [3]. Barr body testing is also efficient in identifying transplanted porcine Retinal pigment epithelium (RPE) in the opposite gender [4]. Cancer detection in the uterine cervix is also possible based on the number of Barr bodies detected. The incidence of double Barr bodies is higher among carcinoma and carcinoma in situ conditions [5]. Sex determination to eliminate the controversy that has arrived in Olympics since Hitler's time in 1936 regarding gender and sex determination has been made compulsory by Nude parades and Gynaecological tests earlier. This has been replaced with Barr body testing since 1968 in the Olympics [6]. In most criminal investigations and forensic analysis, establishing the identity of gender is crucial for progressive investigation and avoiding tampering with evidence.

Sex can be determined either by morphological analysis or molecular analysis. The morphological analysis includes hard or soft tissue analysis, which involves the tooth dimension or reconstruction of post-mortem dental profiles [7], morphological traits of the skull and chelioscopy, and Rugoscopy of soft tissue analysis. The molecular analysis includes – DNA extraction from dental pulp, cartilage, hair, skin, and presences of Barr bodies, F-bodies, and SRY genes [8]. Along with the Barr bodies, Davidson bodies are helpful in finding the gender of an individual; it is seen on neutrophils resembling drumsticks [9]. The presence of the Barr body makes relatively smaller nuclear size in females due to its condensed form [10]. Sex determination can be made easy using a basic compound microscope and the Nissl method of staining with cresyl violet and thionin stains [2].

Instead of using PCR and fluorescent assays, sex estimation through Barr body detection can be performed through basic staining procedures, which are relatively less costly and time-effective. So far, studies have been performed on dental, buccal, amniotic fluid and blood, skin, epithelial cells of urinary residues, vaginal cells, and nail samples with a wide variety of stains. To visualize Barr Bodie's presence, any dye that can stain negatively charged particles, i.e., DNA, is used to date. The majority of the studies have been performed using stains such as Papanicolaou, Arcidine orange, carbol fuchsin, haematoxylin and eosin, diamond fuschin, May-Grünwald Giemsa (MGG), Methylene blue, Aceto-orcein in Barr body detection and sex determination.

Aims and Objectives:

Neither the studies have used saffranine in Barr body detection. Saffranine is a positive stain that can easily stain DNA, and it is readily available in any biochemical laboratory. Using Saffranine in Barr body detection is cost-effective and will have potent to save time. The current aim of the study is to assess the sex estimation efficacy of Saffranine through cytological assessment of Barr bodies and compare the efficacy of Saffranine and Methylene blue stains in detecting Barr bodies.

Experimental section:

This is a prospective cross-sectional study design. A total of 60 healthy individuals (30 males and 30 females) were considered samples based on a simple random sampling technique. Preprocedural rinsing is performed by asking all the subjects to rinse their mouth with distilled water to prevent contamination with already taken consumables and avoid mucus or debris in the sample. Any subject having oral infections, ulcers, or diagnosed to have any other systemic conditions or social history of smoking or tobacco chewing has been excluded from the study. This study is performed in accordance with the declaration of Helsinki and has taken informed consent from each participant.

The buccal scrapes were collected with a sterile wooden toothpick, and the scrapings were suspended onto the sterile and dry microscopic slide. Each sample is organized into 2 smears, one is treated with safranin, and another smear is treated with methylene blue. The excess stains were blotted out with a Whatman filter paper and allowed the slides to air dry. The slides are observed under 40X magnification of the Binocular compound microscope. All the samples were blindfolded and analyzed for cytological assessment without knowing the predetermined gender of the sample collected.

The following strategy is used to grade the presence of Barr bodies:

a) No stain/absence of staining as 0
b) Buccal cell is stained properly but nucleus not seen as 1
c) Cell Stained properly, but Barr body is not seen as 2
d) Showing shrunken cells along with the presence of Barr body as 3
e) Shows the presence of Barr bodies but cannot be very distinctly identified as 4
f) Shows the distinct presence of Barr bodies (i.e., seen in more than 8 cells on the slide) as 5.

Samples graded between 3 to 5 scores and with> 2 Barr bodies were recorded as female. On the other hand, samples with ≤ 2 are considered males [11, 12]. Cytological estimation of sex was then compared with the known sex of subjects.

Statistical analysis:

Data were summarized into frequencies and proportions using descriptive statistics. According to the Kolmogorov-Smirnov test, neither the Saffranine nor methylene blue staining scores were normally distributed. Thus, non-parametric tests were used for further analysis; the Mann-Whitney test was applied to compare two groups, and sensitivity and specificity for both staining procedures were calculated. Results were considered statistically significant when P-value was < 0.05. Statistical analyses were performed using the Statistical Program for Social sciences (IBM SPSS Statistics 22 Version).

Results:

A total of n=60 buccal samples were collected, and two smears were constructed from each sample. Of 120 smears prepared, 60 (30 male and 30 female) were stained with Saffranine and another 60 with methylene blue (see graph 1).

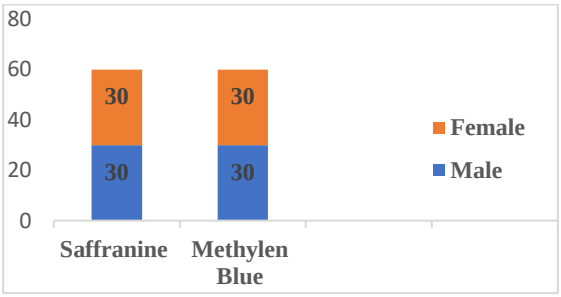

Figure 1: Bar graph describing the sample distribution across stains and gender.

Barr bodies appear as darkly stained and lying intact at the periphery of nuclear membrane. (See figure 2 & 3)

eISSN1303-5150

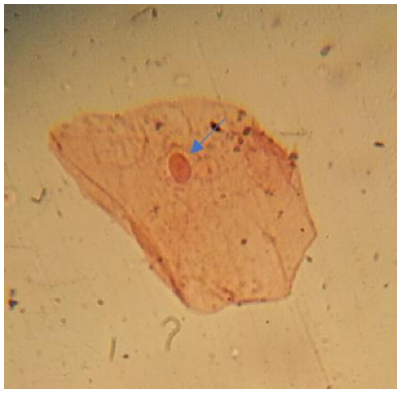

Figure 2: Buccal cell stained with Saffranine showing positive for presence of Barr body indicated by arrow mark

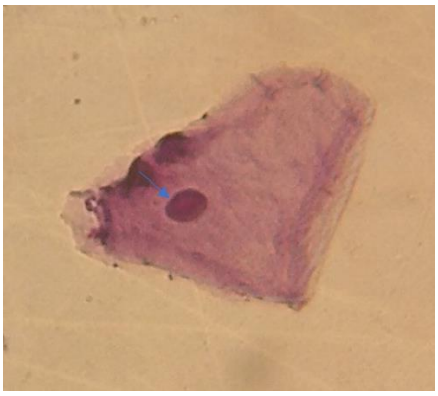

Figure 3: Buccal cell stained with Methylene Blue showing positive for presence of Barr body indicated by arrow mark

Among females, 96.7% of them stained with saffranine showed well distinct Barr bodies seen in more than 8 cells and a tiny percent, i.e., 3.3%, showed indistinct Barr bodies presence. (See figure 4)

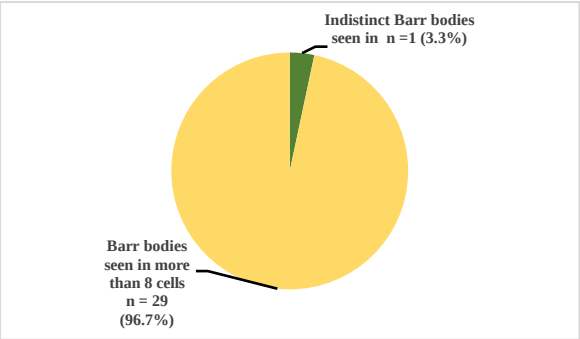

Figure 4: Staining score frequency using Saffranine in females

When the same female smears were stained with methylene blue, 63.3% of smears showed indistinct Barr bodies, and nearly one-third of them showed distinct Barr bodies in more than 8 cells. A tiny proportion (6.7%) have shown negative for Barr bodies. (See figure 5)

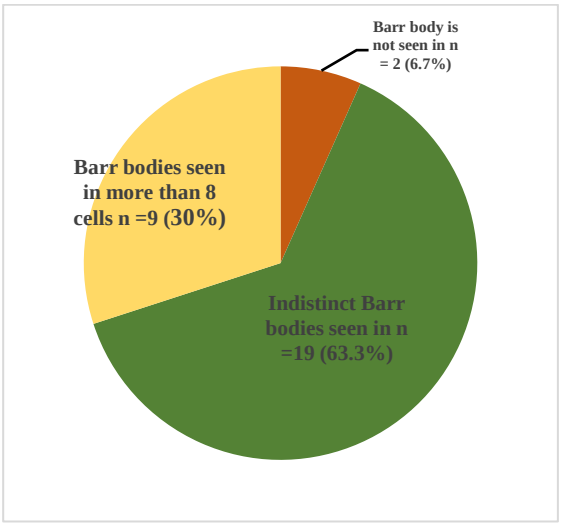

Figure 5: Staining score frequency using Methylene blue in females

On the other hand, among males, 93.3% of them stained with saffranine showed an absence of Barr bodies and 6.7% showed well distinct Barr bodies seen in more than 8 cells. (See figure 6)

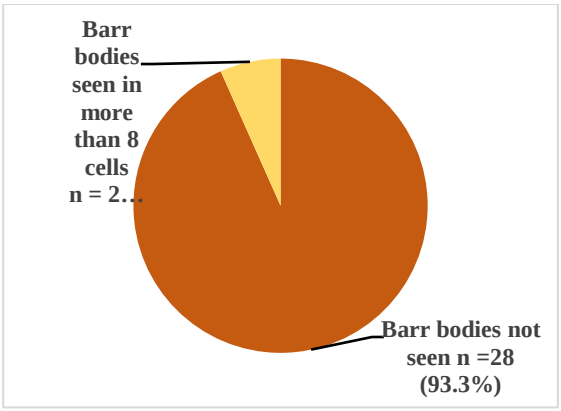

Figure 6: Staining score frequency using Saffranine in males

When the same male smears were stained with methylene blue, 93.3% of smears showed no Barr bodies and indistinct Barr bodies by 6.7%. (See figure 7)

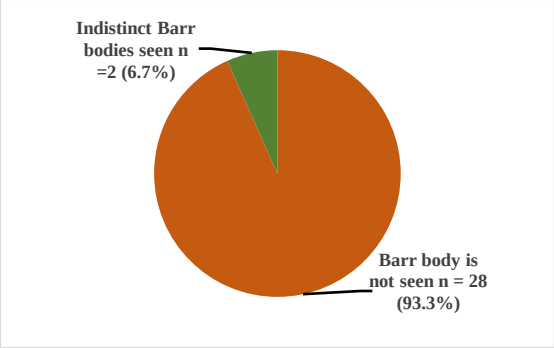

Figure 7: Staining score frequency using Methylene blue in males

Overall, the staining score is different among the stains, but the judgment of gender is similar among both the stains. The staining score is higher among females (Median =5) (from hereafter median called as Md) when the smears are stained with Saffranine with IQR (Inter Quartile Range) (5-5). On the other hand, the staining score is (Md =4) among the same sample when stained with Methylene blue with IQR (5-4). The staining scores among males are similar to both stains. (See table 1)

Table 1. Saffranine and Methylene Blue staining scores.

	Saffranine		***Methylene Blue***	
	Female	Male	Female	Male
Median staining score	5	2	4	2
Inter Quartile Range (Q3-Q1)	5 - 5	2 - 2	5 - 4	2 - 2

Our Mann-Whitney U test has concluded that Saffranine has a better staining performance with (U = 31.0) (Overall Md =5) and P < 0.001 than Methylene Blue stain among females with (U = 51.0) (Overall Md =3) and P < 0.001. Correspondingly, the sensitivity and specificity for sex estimation using Safranine are 100% and 93.3%, respectively, whereas sensitivity and specificity using Methylene blue are 93.3% and 93.3%. Overall, it is conspicuous that Saffranine has superior staining performance and, thereby, sex estimation efficacy than Methylene blue.

Discussion:

Barr bodies show a highly specific intranuclear distribution, dividing preferably in a plane parallel to cell growth [13]. The number of Barr bodies depends on the degree of ploidy of the cells and not just on the number of X chromosomes. In diploid cells, one Barr body will be seen, and in triploid cells, the number of Barr bodies could be one or more than one.[14] The number of arm folds of the X chromosome increases during condensation of Barr Bodies [15]. Structural changes are seen during Barr body formation, leading the telomere closer to the nuclear membrane [16]. Bipartite Barr bodies are seen in both Xq isochromosome and isodicentric chromosomes. The Barr body formation is studied earlier using a fluorescence microscope and heterochromatin markers [17]. Barr body recognition is also made using ultrastructure studies on the Xi chromosome [18]. Barr bodies have surface antigens that might be engaged in X chromosome inactivation [19]. The antibodies against these surface antigens are used in identifying the molecular basis of X chromosome inactivation [20].

Barr body testing has a diagnostic value in infertility. 17% of patients (out of 68 patients) in a study have shown abnormal sex chromatin associated with primary amenorrhea and a higher incidence of Turner syndrome [21]. X-linked diseases, neoplastic diseases, recurrent pregnancy loss, and trisomy risks are associated with non-random X-chromosome inactivation (XCI). Skewing of XCI is seen predominantly among XCI after 30 years of age which is possible due to hematopoietic stem cell senescence or loss of methylation leading to reactivation of X-chromosomes, especially in tumor cells [22]. A decrease in the number of Barr bodies is associated with aortic dissection in Turner's syndrome leading to death in a 24-year-old female. There were no specific symptoms except for fainting and cervicodynia 1 day before the death. The post-mortem report revealed the hemopericardium

was caused by a rupture of ascending aorta by thoracic aortic dissection, and weakness was due to medial cystic necrosis of the aorta. Internal examinations have revealed funicular ovaries and coarctation of the aorta. A possible (45, X/46XX) was suspected [23]. The genotoxic effects of pesticide usage can also be determined using cytological assessment of Barr bodies [24].

Accurate computing of Barr bodies in females and y bodies in males can be made quick, easy, and efficient using atebrin-stained hair roots under a fluorescent microscope. Both Barr bodies and Y bodies can be seen simultaneously in Klinefelter's patients [25]

Although sophisticated and highly specific procedures are available to study DNA and Barr bodies' ultrastructure, we have used basic staining procedures for easy cytological assessment of Barr bodies and gender determination.

In a study, diagnostic performance for gender determination is assessed through observing Barr bodies and F bodies from dental pulp tissues. It was noted that gender determination from pulpal tissue is feasible for up to 7 weeks. The percentage of Barr bodies and F bodies gradually decreases with an increase in the time interval [26]. In the current study, immediate cytological assessment is performed by examining samples immediately with no incubation.

The DNA-drawn content will vary depending on the procedure used for harvesting the sample. liquid saliva contains 1000 – 10,000 ng/ml, Oral swabs contain 100 – 1500 ng/swab whereas urine contains 1-20 ng/ml [27]. In a comparison between methods of DNA collection, sample collection through cytology brushes proved to give more qualitative and quantitative results for the DNA Analysis [28].

In a study to assess reliability in using buccal samples, Buccal Barr bodies for sex determination showed 24% and 84% sensitivity and specificity, respectively, with 54% accuracy while using PAP stain [29]. The current study used buccal scrapings using sterile wooden toothpicks, which are ample enough to visualize Barr bodies.

Close to 2 male samples have shown the presence of Barr bodies using either stain. In a similar study, 1.14% of buccal mucosal cells in males and 39.29% of buccal mucosal cells in females visualized Barr bodies' presence when stained with Papanicolaou's (PAP) [30]. Barr bodies are usually seen in females. However, few males will have extra X chromosomes. It is also known that multiple X syndromes have been associated with defective genes causing psychopathic disorders. 60% of the samples collected from jail inmates (males) have tested positive for bar bodies [31].

The assessors were blindfolded, i.e., they were not aware of gender in reality for given collected samples. This procedure is followed in our study to remove the anticipation of Barr bodies presence for a sample, thereby reducing the chance of bias error.

In a comparative study between Aceto-orcein (AO) and Papanicolaou's inefficacy of sex determination using Barr bodies, the Range of Barr bodies seen by Aceto-orcein is higher among both the genders. The sensitivity and specificity for Aceto-orcein are 98.3% and 95% for PAP [32]. In another similar comparative study, Aceto-orcein showed higher staining accuracy and less staining time [33].

In another comparative study between Acridine orange and Papanicolaou's stain in sex determination using Barr bodies in buccal smears, the range of Barr bodies seen in both genders is higher using Acridine orange. The overall sensitivity of Acridine Orange is 97.9%, and the PAP stain with 98% [34]. In a similar study to determine sex determination from Barr bodies using Giemsa stain and methylene blue, Giemsa stain showed 98% accurate result compared to 94% by methylene blue among buccal samples [35].

A comparative study is performed to assess the accuracy and efficacy of sex determination among stains that are simple to use and provide strong contrast. Among females' positivity to Barr bodies is 64% stained by Acridine orange, 46.14% by Papanicolaou's stain, and Carbol fuchsin with 8.68% [36].

Using Giemsa stain, buccal epithelial cells showed 20-50% positivity with a mean of 40.14 % ± 6.59 % among 12 to 60 years females [9]. Papanicolaou stain seemed to be better with less time in 45.14% of cases detected with Barr bodies compared to 8.68% using Carbol

Fuschanin stain [37]. Pap stain is relatively easier and faster to process Barr body visualization, especially in field studies with clear cellular demarcated boundaries compared to May-Grünwald Giemsa (MGG) stain [38]. However, compared to haematoxylin and eosin (H & E) and PAP stains, samples exposed to 200°C and younger age have clear visibility using H and E than PAP [39]. In a similar comparative study for sex determination between Alkaline methylene blue and H& E stain, 88% of accuracy is seen through Alkaline Methylene blue and 80% through H & E stain [40].

Correspondingly, our study used Saffranine stain in sex determination for the first time and has shown better staining scores among both genders. Our study showed sex estimation efficacy of 100% and 93.3% sensitivity and specificity, respectively, using buccal samples through Saffranine stain and 93.3% sensitivity and specificity using methylene blue.

Conclusion:

Visualising Barr bodies have a significant application in multifaceted research domains. Basic staining procedures can be employed in determining the sex of an individual, which are reliable, fast, accurate, and cost-effective. It is established that Saffranine can be used in gender determination with higher efficacy compared to Methylene blue using buccal samples.

Acknowledgment:

We are grateful to our teacher and great motivator, Late Dr. Rishi Bhardwaj.

Conflict of Interest:

The authors have no financial or non-financial conflict of interest.

List of Abbreviations used:

IQR = Inter Quartile Range
Md = Median
PAP = Papanicolaou
AO = Aceto-orcein

Funding Sources:

No funding was provided to this study

Authors contributions:

Vataparthi Pravallika – Conceptualization and design of research, data collection, Analytical interpretation, critical revision and final approval
Manisai Koduri – Design of research, data analysis and interpretation, critical revision and final approval
Sony Gunaganti – Design of research, data analysis and interpretation, critical revision and final approval
Lakshmi Panicker – Conceptualization and design of research, critical revision and final approval

Corresponding Author:

Ms Vataparthi Pravallika
MSc Forensic Science
Assistant Professor
Department of Forensic Science
School of Paramedics and Allied Health Science
Centurion University of Technology and Management, Andhra Pradesh, India.
Email id: pravallikav@cutmap.ac.in

References:

1) 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
2) BARR ML, BERTRAM EG. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature. 1949 Apr 30;163(4148):676. doi: 10.1038/163676a0. PMID: 18120749.
3) Bhardwaj, Nandini & Bhat, Nitish & Gupta, Hitesh & Puri, Abhiney & Nangia, Rajat & Kaur, Rashmeet. International Journal of Current Medical and Pharmaceutical Research Barr Bodies-A Spoonful of Sex Chromatin. 2020;10.24327/23956429.ijcmpr202001838.
4) Jones Z, Tezel TH, Zarbin MA. Definitive identification of transplanted porcine retinal pigment epithelium (RPE) with barr body staining. Invest Ophthalmol Vis Sci 1997;38(4).

5) Nishiya I, Ishizaki Y, Sasaki M. Nuclear DNA content and the number of Barr bodies in premalignant and malignant lesions of the uterine cervix. Acta Cytol. 1981 Jul-Aug;25(4):407-11. PMID: 6945007.
6) Ritchie R, Reynard J, Lewis T. Intersex and the Olympic Games. J R Soc Med. 2008 Aug;101(8):395-9. doi: 10.1258/jrsm.2008.080086. PMID: 18687862; PMCID: PMC2500237
7) Srivastava A, Kumar A, Kumar A, Srivastava A. Mass Disaster: Identification of Victims with Special Emphasis on Dental Evidences. Journal of Indian Academy of Forensic Medicine. 2015;37(2):190.
8) 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
9) R. Shankar1, P. B. Master2, L. C. Obulesu3, and Y. K. C. Rangiah4. Determination of sex, medico legal importance of sex, Barr bodies & Davidson bodies, sex chromatin & inter-sex. A Study Sex Chromatin Form Buccal Smear. no. 9244, 2015.
10) Mittwoch U. Barr Bodies and their Relation to Nuclear Size. Cytogenetic and Genome Research. 1964;3(1):62-74.
11) Nagamori H, Ohno Y, Uchima E, Kajiwara M, Nakazato M, Une Y, Takeda K. Sex determination from buccal mucosa and hair root by the combined treatment of quincrine XXX staining and fluorescent Feulgen reaction using a single specimen, Forensic Sci Int, 1986; 31: 119 – 128.
12) Soe Htun, Kyaw & Aung, Ye & Zaw, Thein & Tin, & Hlaing, Tin. Gender Determination from Barr Bodies Using Giemsa and Methylene Blue Stains in Buccal Mucosal Smears. 2017;10.13140/RG.2.2.33685.58080.
13) Belmont AS, Bignone F, Ts'o PO. The relative intranuclear positions of Barr bodies in XXX non-transformed human fibroblasts. Exp Cell Res. 1986 Jul;165(1):165-79. doi: 10.1016/0014-4827(86)90541-0. PMID: 3709685.
14) MITTWOCH U, ATKIN NB, ELLIS JR. BARR BODIES IN TRIPLOID CELLS. Cytogenetics. 1963;2:323-30. doi: 10.1159/000129789. PMID: 14164309.
15) Butler MG, Joseph GM, Dev VG. Metaphase chromosome folds and X-inactivation. Am J Med Genet. 1987 Mar;26(3):565-8. doi: 10.1002/ajmg.1320260309. PMID: 3565471; PMCID: PMC5494697.
16) Walker CL, Cargile CB, Floy KM, Delannoy M, Migeon BR. The Barr body is a looped X chromosome formed by telomere association. Proc Natl Acad Sci U S A. 1991 Jul 15;88(14):6191-5. doi: 10.1073/pnas.88.14.6191. PMID: 1712482; PMCID: PMC52048.
17) Chadwick BP. Characterization of chromatin at structurally abnormal inactive X chromosomes reveals potential evidence of a rare hybrid active and inactive isodicentric X chromosome. Chromosome Res. 2020 Jun;28(2):155-169. doi: 10.1007/s10577-019-09621-1. Epub 2019 Nov 27. PMID: 31776830; PMCID: PMC7245565.
18) Rego A, Sinclair PB, Tao W, Kireev I, Belmont AS. The facultative heterochromatin of the inactive X chromosome has a distinctive condensed ultrastructure. J Cell Sci. 2008 Apr 1;121(Pt 7):1119-27. doi: 10.1242/jcs.026104. Epub 2008 Mar 11. PMID: 18334550.
19) Hong B, Reeves P, Panning B, Swanson MS, Yang TP. Identification of an autoimmune serum containing antibodies against the Barr body. Proc Natl Acad Sci U S A. 2001 Jul 17;98(15):8703-8. doi: 10.1073/pnas.151259598. Epub 2001 Jul 3. PMID: 11438711; PMCID: PMC37499.
20) Hong B, Reeves P, Panning B, Swanson MS, Yang TP. Identification of an autoimmune serum containing antibodies against the Barr body. Proc Natl Acad Sci U S A. 2001 Jul 17;98(15):8703-8. doi: 10.1073/pnas.151259598. Epub 2001 Jul 3. PMID: 11438711; PMCID: PMC37499.
21) Pansegrau Dg, Peterson Re. Improved Staining of Sex Chromatin. Ninety-Seven And Six Tenths Percent of Female Buccal Cells Chromatin-Positive. Am J Clin Pathol. 1964 Mar;41:266-72. doi: 10.1093/ajcp/41.3.266. PMID: 14130302
22) Hatakeyama C, Anderson CL, Beever CL, Peñaherrera MS, Brown CJ, Robinson WP. The

dynamics of X-inactivation skewing as women age. Clin Genet. 2004 Oct;66(4):327-32. doi: 10.1111/j.1399-0004.2004.00310.x. PMID: 15355435.

23) Mimasaka S, Ohtsu Y, Tsunenari S, Matsukawa A, Hashiyada M, Takahashi S, Funayama M. Sudden death of a young woman due to aortic dissection caused by Turner's syndrome. Pathol Int. 2007 Apr;57(4):219-23. doi: 10.1111/j.1440-1827.2007.02084.x. PMID: 17316418.

24) Pastor S, Creus A, Xamena N, Siffel C, Marcos R. Occupational exposure to pesticides and cytogenetic damage: results of a Hungarian population study using the micronucleus assay in lymphocytes and buccal cells. Environ Mol Mutagen. 2002;40(2):101-9. doi: 10.1002/em.10100. PMID: 12203402.

25) Fran cois J, Mation-van Leuven MT, Acosta J. Male and female sex determination in hair roots. Clin Genet. 1971;2(2):73-7. doi: 10.1111/j.1399-0004.1971.tb00258.x. PMID: 4107305.

26) Khorate MM, Dhupar A, Ahmed J, Dinkar AD. Gender determination from pulpal tissue. J Forensic Dent Sci. 2014 May;6(2):107-12. doi: 10.4103/0975-1475.132540. PMID: 25125918; PMCID: PMC4130012.

27) Chatzinikolaou, F. & Dressler, M.L. & Enache, Alexandra & Belei, V. & Matei, V. Collection and preservation of evidence for human identification by DNA analysis. 2002;10. 215-221

28) Mulot C, Stücker I, Clavel J, Beaune P, Loriot MA. Collection of human genomic DNA from buccal cells for genetics studies: comparison between cytobrush, mouthwash, and treated card. J Biomed Biotechnol. 2005;2005(3):291-6. doi: 10.1155/JBB.2005.291. PMID: 16192688; PMCID: PMC1224694.

29) Kaur, Navdeep & Sidhu, Ruhi & Chandra, Sunira & Taneja, Neeraj. Buccal Barr Bodies: Accuracy and Reliability in Sex Determination. Saudi Journal of Oral and Dental Research. 2. 2017.

30) 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.

31) Kulkarni MK, Somannavar PD, Kotrashetti V, Nayak R, Hosmani J, Babji D. Do the presence of Barr bodies in male jail inmates indicates criminality: A pilot study. J Oral Maxillofac Pathol. 2016 Jan-Apr;20(1):11-5. doi: 10.4103/0973-029X.180906. PMID: 27194855; PMCID: PMC4860910.

32) Datar U, Angadi PV, Hallikerimath S, Kale AD. Cytological assessment of Barr bodies using aceto-orcein and papanicolaou stains in buccal mucosal smears and their sex estimation efficacy in an Indian sample. Acta Cytol. 2013;57(5):516-21. doi: 10.1159/000353216. Epub 2013 Sep 7. PMID: 24021272.

33) Ashok, Sahana. Aceto-orcein squash technique for Barr body detection. RGUHS Journal of Dental Sciences. 9. 2019;10.26715/rjds.9_1_3.

34) N. Aziz, Bg. Prasad, K. Arathi, F. Britto, B. Chethan Aradhya, and B. Abdulrashid,. Efficacy of acridine orange and papanicolaou stains in sex determination using barr bodies in buccal smears: A comparative study," Int. J. Prev. Clin. Dent. Res., vol. 6, no. 1, p. 7, 2019, doi: 10.4103/INPC.INPC_11_19.

35) Soe Htun, Kyaw & Aung, Ye & Zaw, Thein & Tin, & Hlaing, Tin. Gender Determination from Barr Bodies Using Giemsa and Methylene Blue Stains in Buccal Mucosal Smears. 2017;10.13140/RG.2.2.33685.58080.

36) Archana, Talari & Bashamalla, Rithika & Rao, Guttikonda & Sravya, Taneeru & Sivaranjani, Yeluri & Kiran, Madala. Cytological assessment of Barr body in buccal scrapes: A comparative study. Journal of Pierre Fauchard Academy (India Section). 31. 2017;10.1016/j.jpfa.2016.11.006.

37) N. K. Meena, M. Singh, and J. Singh. A comparison study of carbol fuchsin and papanicolaou staining methods for the demonstration and enumeration of barr bodies in buccal smear. Int. J. Appl. Res., Feb. 26, 2021. vol. 2, no. 5, pp. 941–944, 2016.

38) Ayyad SB, Israel E, El-Setouhy M, Nasr GR, Mohamed MK, Loffredo CA. Evaluation of Papanicolaou stain for studying micronuclei in buccal cells under field conditions. Acta Cytol. 2006 Jul-Aug;50(4):398-402. doi: 10.1159/000325980. PMID: 16901002.

39) Murugesan, Ambika & Balakrishnan, Sekar & Kumaresan, I.P. & Jayakumar, Arun & Mohan, Jayashree & Champakesan, Bharath. Cytological Method of Barr Body Expression in Dental Pulp Tissue at Varying Temperature. Journal of Orofacial Sciences. 2018;10. 108-111. 10.4103/jofs.jofs_102_18.

40) Singh, Shruti & Radhika M Bavle, Radhika & Hosthor, Sreelatha & Bopaiah, Shiny & Kumar, Sameer. Special Stains to Disclose Barr Bodies in Buccal Scrape for Gender Determination in Forensic Science. International Journal of Contemporary Medical Research. 4.2015; 777-780.