Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jpfa



Cytological assessment of Barr body in buccal scrapes: A comparative study

Talari Archana *, *Rithika Bashamalla*, *Guttikonda Venkateswara Rao*, *Taneeru Sravya*, *Yeluri Sivaranjani*, *Madala Jaya Kiran*

Department of Oral Pathology & Microbiology, Mamata Dental College, Khammam, Telangana 507002, India

ARTICLE INFO

Article history:
Available online 5 January 2017

Keywords:
Barr body
Carbol fuchsin
Papanicolaou
Acridine orange

ABSTRACT

Introduction: Barr body is a deeply stained condensed chromatin material seen in the nuclei of females, which is taken as a vital tool in the identification and determination of sex of the individual. Various staining techniques can be used for their demonstration. However, staining with carbol fuchsin, papanicolaou and acridine orange were considered appropriate as they produce stronger contrast and are simple to use.

Aim: To determine the sex and also to assess the accuracy and efficacy of special nuclear stains like carbol fuchsin, papanicolaou and acridine orange in staining Barr bodies.

Materials and methods: A total of 300 samples were included in the present study. The smears were collected and subsequently stained with three different special stains of which 100 samples were stained with acridine orange, 100 with papanicolaou and 100 with carbol fuchsin. Stained smears were examined for Barr bodies and also for their staining accuracy and efficacy.

Results and discussion: Barr body was found to be negative in the male samples and positive for female samples with acridine orange yielding 64%, papanicolaou yielding 46.14% and carbol fuchsin 8.68% of Barr-body-positive cells.

Conclusion: On comparison, the efficacy and accuracy for detection of Barr bodies were more with acridine orange followed by papanicolaou and carbol fuchsin.

© 2016 Pierre Fauchard Academy (India Section). Published by Elsevier, a division of RELX India, Pvt. Ltd. All rights reserved.

1. Introduction

Establishing the gender of a person is of paramount importance in forensic science. Various methods have been described for gender determination including the use of craniofacial morphology, tooth dimensions and DNA analysis. Specimen like blood, semen, hair, buccal epithelial cells, fibroblasts of pulp, cervical cells, skin and saliva stains found in various parts of the body or on harmful weapons at a crime prospect as well as at disaster sites can also be used for gender identification.[1]

Techniques like polymerase chain reaction (PCR), karyotyping, fluorescent body (Y chromatin), Davidson body in the polymorphonuclear leukocytes, AMEL identification and Barr bodies (X chromatin) examination through cytological procedures can validate the gender.[2] However, PCR and karyotyping are very expensive and are not feasible for use.[1] Thus, Barr body demonstration for gender determination

* *Corresponding author.*
E-mail address: archana.mdhvn@gmail.com (T. Archana).
http://dx.doi.org/10.1016/j.jpfa.2016.11.006
0970-2199/© 2016 Pierre Fauchard Academy (India Section). Published by Elsevier, a division of RELX India, Pvt. Ltd. All rights reserved.

using exfoliative cytology is considered one of the simplest and easiest methods.[3]

Barr body is a facultative heterochromatic body which is classically seen during interphase as a dark-staining, peripheral nuclear structure in somatic cell nucleus of normal female but absent in males. The distribution of Barr body present in an individual cell by itself when there is more than one X chromosome in the chromosomal structure can be understood by the knowledge of Lyon inactivation hypothesis.[4]

The analysis of Barr bodies in cell nuclei from easily accessible tissues such as buccal mucosa, hair root and blood cells allows provisional description of the sex chromosome status of individuals hermaphroditism, gonadal, and some complicated anomalies, whereas the use of amniotic fluid assists a prenatal sex diagnosis.[5] Various nuclear stains such as haematoxylin and eosin (H and E), thionine, papanicolaou, feulgen, cresyl-violet, giemsa, aceto-orcein, and fluorescent stains like acridine orange can validate Barr bodies.[6] However, special stains such as carbol fuchsin (CF), papanicolaou (PAP) and acridine orange (AO) were appropriate as they produce stronger contrast and are simpler to use.[4]

So, the present study was probably being the first study to determine the sex and also to compare accuracy and efficacy of staining of Barr bodies with three different special nuclear stains like CF, PAP and AO.

2. Material and methods

A total of 100 healthy subjects who visited outpatient department of our college were included in the present study. Among 100 subjects, 50 were males and 50 were females above 18 years of age.

Inclusion criteria:

1. Healthy subjects with no lesions and habits.
2. Age: Individuals with age group of more than 18 years.

Exclusion criteria:

1. Subjects with detrimental habits such as tobacco and alcohol consumption.
2. Age: Individuals with age group of less than 18 years.
3. Subjects with systemic diseases.

2.1. *Method of collection of samples*

After obtaining ethical clearance, the study was undertaken. The subjects were asked to rinse the mouth with mouthwash and then with water. A sterilized spatula was used to scrape the buccal surface of the cheek, where the initial scrapings were cast off, as they may be lodged with micro-organisms and occasionally with food particles. A fresh spatula was then used to collect mucosal cells which were carefully smeared onto three grease-free, graphite-labelled slides. These slides were immediately plunged into a Coplin jar containing 95% ethyl alcohol and were air-dried to make the cells adhere more firmly to the slide and were then stained with three different special staining techniques, namely CF, PAP and AO.

2.2. *Method for processing of samples*

A total of three smears from each individual were collected resulting in 300 samples of which 100 smears were stained with CF, 100 with PAP and other 100 with AO.

For AO staining, the fixed smears were passed through descending grades of alcohol and then rinsed for a few seconds in 1% acetic acid and washed in two changes of distilled water for about 1 min, followed by staining in 0.01% AO for 3 min, de-staining in the phosphate buffer solution for 1 min, differentiated in 0.1 M calcium chloride solution for 30 s to 1 min. Excess calcium chloride was removed by washing with phosphate buffer solution and mounting was done with cover slip using a drop of phosphate buffer solution.

For PAP staining, the smears were fixed in 95% ethyl alcohol for 15–30 min, rinsed in distilled water and stained in Harris's haematoxylin for 4 min. The slides were washed under tap water for 1–2 min, differentiated in acid alcohol, blued in 1.5% sodium bicarbonate and rinsed in distilled water. Then, these were transferred to 70% and then 95% alcohol for a few seconds. After staining in orange G 6 for 1–2 min, these were rinsed in three changes of 95% alcohol for a few seconds each and then stained in eosin azure 36 for 1–2 min. These were rinsed again in three changes of 95% alcohol for a few seconds each. Finally, those were dehydrated in absolute alcohol, cleared in xylol and mounted in dibutyl phthalate xylene.

For CF staining, smears were spread over albumenized slides. They were fixed for 30 min in 95% ethyl alcohol. Then smears were hydrated using 80%, 70%, 50% alcohol in the descending order for about 2–5 min followed by water. The slides were then stained by CF. Then differentiate them in 95% ethyl alcohol. After that, slides were put in absolute alcohol for 1 minute followed by clearing in xylene. Lastly, the slides were mounted in dibutyl phthalate xylene.

The smears stained with PAP and CF were observed under binocular light microscope at 100× magnification while the AO stained smears were observed under a fluorescent microscope at 100× magnification. A total of 100 cells were observed in each slide from which Barr-body-positive cells were counted. Further, a comparison was made between the factors such as number of Barr-body-positive cells, nuclear staining and cytoplasmic staining. The obtained values were tabulated and sent for statistical analysis.

3. Results

In the male samples, Barr-body-positive cells were not observed with any of the 3 different special stains, that is, CF, PAP and AO. In the female samples, Barr-body-positive cells were observed with a percentage 5–14% for CF stain, 14–50% for the PAP stain and 18–72% for AO respectively (Table 1). None of the females showed less than 5% Barr-body-positive cells for CF stain (Fig. 1), 14% for PAP (Fig. 2) and 18% for AO (Fig. 3). The mean percentage of Barr-body positive-cells observed was $9.68 \pm 2.47\%$ for the CF stain and $41.14 \pm 13.76\%$ for the PAP stain and 68.64 ± 24.46 for AO (Fig. 4).

The percentage of nuclear and cytoplasmic staining intensity and nuclear membrane integrity using three different special nuclear stains was calculated and tabulated in Table 2.

Table 1 – Mean values for Barr body using three different special stains.

Barr body percentage by	Number of female samples	Min	Max	Mean	SD
Carbol fuchsin stain	50	5	14	9.68	2.470
Papanicolaou stain	50	14	50	41.14	13.762
Acridine orange stain	50	18	72	68.64	24.46
				$p \leq 0.000^{*}$	

* $p < 0.05$ which is statistically significant.

The difference between mean percentage values was found to be highly significant with a p value <0.000 (Table 1).

4. Discussion

During the embryonic development, females shut off one of their X chromosomes which undergoes random inactivation and condensation.[4] This inactivated X chromosome is fundamentally present in all the somatic cells of female mammals and is called as Barr body as it was first described by Barr and Bertram in 1949. They found a small body present in the nuclei of neurons of female cats but absent in the neurons of male cats.[1] It has a normal size of about 1 μm with an average of 0.7–1.2 μm in section and is characterized as round, oblong, triangular, plano-convex or flattened body lying contiguous to the nuclear membrane internally.[4]

The present study was conducted to evaluate rapidity with reliability of three different special nuclear stains in Barr body demonstration, cytoplasmic & nuclear intensity along with nuclear membrane integrity. The male subjects revealed 0% Barr bodies and females showed 5–14%, 14–50% and 18–72% Barr bodies in buccal mucosal cells using CF stain, PAP stain and AO stain respectively.

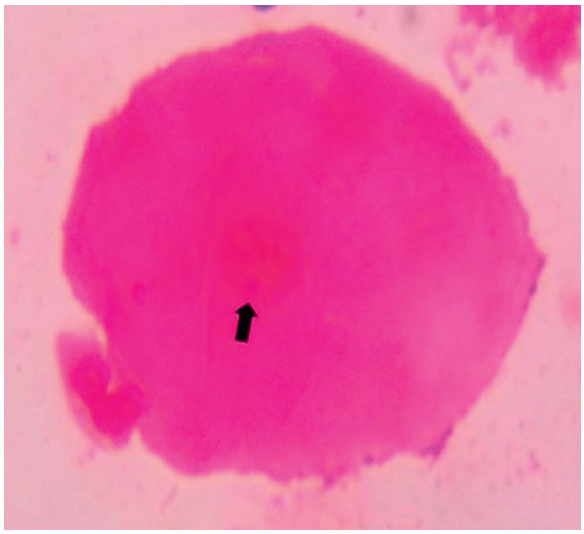


Fig. 1 – Demonstration of Barr body with CF stain under binocular microscope [400×].

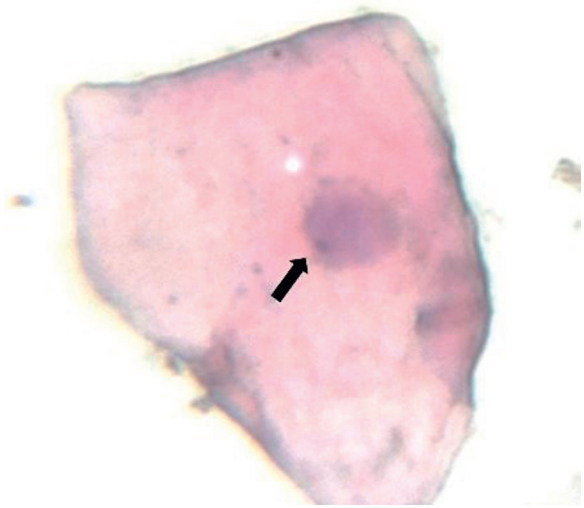


Fig. 2 – Demonstration of Barr body with PAP stain under binocular microscope [400×].

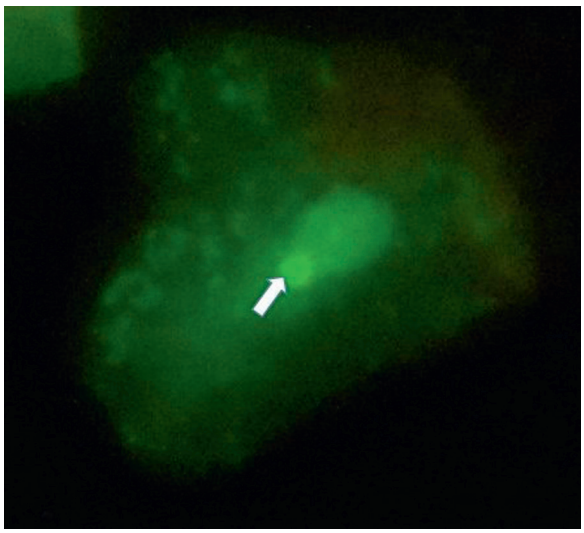


Fig. 3 – Demonstration of Barr body with AO stain under fluorescent microscope [400×].

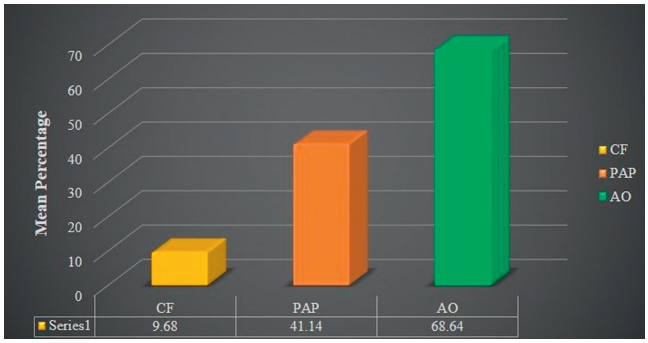


Fig. 4 – Mean percentage of Barr-body-positive cells.

Table 2 – Percentage of nuclear and cytoplasmic staining intensity and nuclear membrane integrity using three different special nuclear stains.

Characteristic features observed	Nuclear staining observed		Cytoplasmic staining observed		Nuclear membrane integrity observed	
Staining characteristics	Light stained	Deep stained	Light stained	Deep stained	Smooth membrane	Rough membrane
Carbol fuschin	15.3%	84.7%	17.2%	82.8%	23.4%	76.6%
Papanicolaou	37.4%	62.6%	87.3%	12.7%	88.2%	11.8%
Acridine orange	57.8%	42.2%	94.6%	5.4%	93.5%	6.5%

Hermann and Davis[7] in 1955 evaluated a total of 100 oral smears for Barr bodies and stated 0–2% incidence of Barr body positive in males and 10% and 32% in females. Reddy et al.[3] in 2012 examined mucosal samples stained with aceto orecin for the finding of Barr-body-positive cells and found out that female sample showed 18–72% cells showing Barr-body-positive cells whereas male samples showed 1–3%. In a study done by Datar et al.[1] in 2013, PAP stain was used in which the range of Barr-body-positive cells was observed as 4–20 in females and 0–5 in males. All the above studies were in contrast to the present study.

However, studies done by Manjula Bhai et al.[8] and Suazo et al.[9] did not report any Barr-body-positive cells in males. Galdames et al.[10] in 2010 conducted a study on histological sections of human dental pulp using H and E stain and found out that the mean of Barr-body-positive cells was 20.4 (SD 0.44) in female samples. There were no Barr-body-positive cells in preparations of male subjects which were in accordance with the present study.

The variations in the incidence of Barr bodies in the different studies can be attributed to:

1. The varied traditions.
2. The types of staining methods used.
3. The diverse criteria of selection of Barr bodies.
4. The differences in methods of fixation and staining protocols.
5. In females the phase of the menstrual cycle.[1]
6. In dead bodies, the post mortem time.[2]
7. The number of samples collected.[4]
8. The shape of the sex chromatin body.[11]

It is assumed that if the Barr body is plump, its demonstration is usually high and it is low, if it is flattened against the nuclear membrane because of difficulty in identifying it. The technical factors like fixatives may vary greatly in their preservation of nuclear details. The formalin may often, but not always produce such homogeneity of the chromatin pattern, that the sex chromatin may be masked. Similarly, the other fixatives may also block the staining of sex chromatin. Fixation in strongly acid fixatives for long periods causes nucleotides to hydrolyze out, so that the staining characteristics will be poor. If the stain used strongly colours the nucleolus, other cell components or bacteria, the sex chromatin will be difficult to be recognized.[11]

Usually, demonstration of Barr body is superior when the nuclear staining is light, because in light nuclear background the deeply stained Barr body is seen prominently.[4] In the present study, PAP and CF gave violet and dark pink staining to the nucleus respectively, with CF displaying more intensity than PAP resulting in less appreciable Barr bodies in CF compared to PAP. AO gave still lighter intensity to the nucleus than PAP resulting in light nuclear background in which the deeply stained Barr body was seen prominently as apple green birefrengence.

Demonstration of nuclear membrane is noble when the nuclear membrane is preserved and smooth.[4] In the present

Table 3 – Differences between the special stains used in the present study.

Carbol fuchsin	Papanicolaou	Acridine orange
Time consuming	Time consuming	Time saving
Screening more samples – difficult	Screening more samples – difficult	More samples can be screened at an ease
Chances of misinterpretation as Barr body on contamination with bacteria	Chances of misinterpretation as Barr body on contamination with bacteria	Bacteria are not stained – as it is a DNA-specific stain
Accuracy and efficacy on staining – less	Accuracy and efficacy on staining are more than CF	Accuracy and efficacy on staining are more than PAP
Special equipment is not necessary	Special equipment is not necessary	Special equipment is necessary
Inexperienced hands can also perform the procedure	Inexperienced hands can also perform the procedure	Inexperienced technologists can misinterpret smears
Cost effective	Cost effective	Expensive
Chances of fading of stained smears is less	Chances of fading of stained smears is less	Chances of fading of stained smears is more

study, percentage of nuclear membrane integrity for AO was more, followed by PAP and CF signifying AO as a better stain.

Visualization of Barr body is also improved when there is light cytoplasmic staining for the cell.[4] In our study, CF stain gave dark pink staining to the cytoplasm, PAP stain gave eosinophilic colour and AO pale green colour ensuring the light cytoplasmic staining of AO as a better stain.

On comparison, the AO was better than PAP and CF stain. This is the first study conducted in the literature so far comparing three different nuclear stains for demonstration of Barr bodies, nuclear and cytoplasmic intensity and nuclear membrane integrity. Various differences encountered between the three special stains in the study, that is, CF, PAP and AO are listed in Table 3.

5. Conclusion

Sex determination using Barr bodies in buccal scrapes is a simple method providing about 95–98% accuracy which can be employed as a significant adjunct to other approaches of sex determination. However, in borderline cases, it is essential to utilize other more intricate methods. In inference, in the study conducted, the special nuclear stain acridine orange stained better when compared to usual carbol fuchsin stain and papanicolaou stain signifying its importance in Barr body study.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. 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;57:516–521.
2. Khanna KS. Efficacy of sex determination from human dental pulp tissue and its reliability as a tool in forensic dentistry. *J Int Oral Health*. 2015;S:1–7.
3. Reddy DS, Sherlin HJ, Ramani P, Prakash PA. Determination of sex by exfoliative cytology using acridine orange confocal microscopy: a short study. *J Forensic Dent Sci*. 2012;4:66–69.
4. Meena DNK, Singh DM, Singh DJ. A comparison study of carbol fuchsin and papanicolaou staining methods for the demonstration and enumeration of barr bodies in buccal smear. *IJAR*. 2016;2:941–944.
5. Lyon MF. X-chromosome inactivation and human genetic disease. *Acta Paediatr*. 2002;91(suppl):107.
6. Mittwoch U. Sex chromatin. *J Med Genet*. 1964;1:50–76.
7. Davis AM, Herrmann W. The determination of chromosomal sex by oral smears. *Yale J Biol Med*. 1956;29:69–74.
8. Manjula Bhai KH, Yadwad BS, Patil PV. A study of Barr bodies in Indian, Malaysian and Chinese subjects. *J Forensic Med Toxicol*. 1997;14:9–13.
9. Suazo GI, Roa HI, Cantin LM. Sex chromatin in dental pulp: performance of diagnosis test and gold standard generation. *Int J Morphol*. 2010;28:1093–1096.
10. Galdames IS, Flores A, Roa I, Cantin M, Zavando D. Sex determination by observation of Barr body in teeth subjected to high temperature. *Int J Morphol*. 2011;29:199–203.
11. Verma U, Chowdhary DS, Chhabra S. Sex chromatin positive cells in the buccal smears of normal newborn females. *Int J Biol Med Res*. 2013;4:3317–3319.