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

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.

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

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, destaining 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.

Please cite this article in press as: Archana T, et al. Cytological assessment of Barr body in buccal scrapes: A comparative study, *J Pierre Fauchard Acad (India Sect)*. (2017), http://dx.doi.org/10.1016/j.jpfa.2016.11.006