Gender Determination in Deciduous Pulp Tissue using Barr Bodies by Comparing Feulgen Stain, Acridine Orange, and Papanicolaou Stain

Yoithapprabhunath Thuckanaickenpalayam Ragunathan, Aarthi Arumugam, Ganapathy Nalliappan, Dineshshankar Janardhanam, Illayaraja Vadivel, Thangadurai Maheswaran

Department of Oral and Maxillofacial Pathology, Vivekanandha Dental College for Women, Thiruchengodu, Tamil Nadu, India

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Abstract

Background: Age, sex, stature, and ethnic background are considered a main attributes of biological identity of the individual, which are also called the "Big Four" in forensic. Human identification is of chief importance, in both legal and humanitarian purpose. Teeth can remain virtually unaffected and survive long after other soft tissue and skeletal tissues were destroyed. Hence, its implication in personal identification was gained popularity and high accuracy. **Aim and Objective:** The aim and objective of the study were to determine the reliability of gender determination from deciduous dental pulp tissue by comparing Feulgen stain, acridine orange, and Papanicolaou stain. **Materials and Methods:** This study was carried out on 25 samples, out of which 14 female teeth and 11 male teeth. The pulp was collected from the teeth undergoing pulpectomy. Then, the pulp tissue was transferred to the centrifuge tube containing Carnoy's fixative. After centrifugation, the cells were collected and stained using Feulgen stain, acridine orange, and Papanicolaou stain. **Results:** The reliability of gender determination is significant, with the female shows 100% positive results and the male shows negative. The Papanicolaou stain shows better nuclear morphology while comparing with Feulgen and acridine orange. **Conclusion:** Hence, gender determination can be identified using deciduous dental pulp.

Keywords: Barr bodies, dental pulp, gender determination

Submitted: 27-Dec-2022; **Revised:** 24-Apr-2023; **Accepted:** 16-May-2023; **Published:** 31-Jul-2023

Introduction

Federation Dentaire Internationale defined forensic odontology as "that branch of dentistry which, in the interest of justice, deals with the proper handling and examination of dental evidence, and with the proper evaluation and presentation of dental findings." It primarily involves the unique features present within the dental structures as a tool for identification. Determination of sex is most important in forensic odontology with bodies mutilated beyond recognition due to a major mass disaster. Recently, DNA analysis in forensic have gained attraction, but it is expensive and timeconsuming. This prompted us to look for other methods of dental identification, which will be costeffective yet reliable.[1] Sex identification becomes the most intriguing, complex, and sometimes controversial challenge.[2] Human identification is one of the major research in forensic science because it deals with establishing human identity. Tooth enamel is the hardest structure in the body; hence, it protects the structures inside it and provides a way for sex determination in forensic with the morphology and content of the soft tissue. Gender determination using dental pulp or dentin of tooth can also provide criminal investigators with useful intelligence and can aid in identifying the missing persons and disaster victims. Age and sex of the person can be determined in forensic odontology.[2] X and Y chromosome in its inactivated form is used as a tool in identifying the gender of the individual. X chromatin in its inactivated form is present as a mass against

Address for correspondence: Dr. Yoithapprabhunath Thuckanaickenpalayam Ragunathan, Department of Oral and Maxillofacial Pathology, Vivekanandha Dental College for Women, Elayampalayam, Namakkal, Thiruchengodu - 637 205, Tamil Nadu, India.
E-mail: yoitha.dentist@gmail.com

Access this article online

Quick Response Code:

Website: https://journals.lww.com/DMRS

DOI: 10.4103/dmr.dmr_48_22

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How to cite this article: Ragunathan YT, Arumugam A, Nalliappan G, Janardhanam D, Vadivel I, Maheswaran T. Gender determination in deciduous pulp tissue using Barr bodies by comparing Feulgen stain, acridine orange, and Papanicolaou stain. Dent Med Res 2022;10:41-3.

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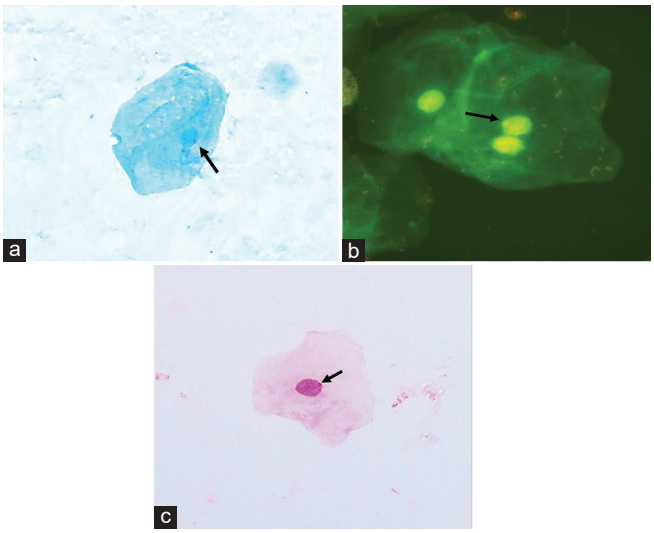


Figure 1: Pictures of Barr bodies under three different stains. (a) Barr bodies under Papanicolaou stain. (b) Barr bodies under Acridine orange. (c) Barr bodies under Feulgen stain

the nuclear membrane in females known as Barr body as it was first named by Barr and Bertram (1949).[3] Females with Barr bodies of 40% are considered chromatin positive and males without Barr bodies are considered chromatin negative.[4] Thus, the aim of this study is to identify the sex of the individual using dental pulp by comparing Feulgen stain, acridine orange stain, and Papanicolaou stain.

Materials and Methods

This prospective study was conducted on the specimens who were collected from the outpatient ward of our dental college.

Inclusion criteria

- Retained deciduous teeth
- Teeth undergoing pulpotomy procedure.

Exclusion criteria

- Grossly destructed teeth.
- Nonvital tooth.

Methodology

This study was conducted after obtaining ethical clearance from our institutional ethical committee. The total sample size is 25. It was calculated using:

$$n = (Z_{1-\alpha/2}\,\sigma/d)^2$$

The pulp was obtained from the teeth undergoing pulpectomy procedure. The pulp tissue was taken into the dry and clean conical centrifuge tubes containing 5 ml of fixative (3 methanol: 1 glacial acetic acid) and left for about half an hour to 24 h for the fixation of the pulp cells. Then, the pulp tissue was crushed/teased using the glass rod to isolate the pulp cells. After centrifugation of the sample at 1000 rpm for 10 min, a suspension was obtained. Then, the supernatant solution was discarded, leaving the pellet in the centrifuge tube. The pellet was resuspended by adding 5 ml of fresh fixative, and the process was repeated thrice till a clear suspension of the pulp cells was obtained.[2] Thin smears were prepared on slides of 1 mm thickness by the air-drying method. Smears made from each suspension of the specimen were stained with Feulgen stain, acridine orange, and Papanicolaou stain.

After staining, the slides were mounted using DPX mountant with coverslip. Then, 50 cells were taken and observed for Barr bodies in each slide in the zigzag manner. For Feulgen stain and Papanicolaou stain, we used light microscope, and for acridine orange, we observed using fluorescent microscope. In Feulgen stain, the Barr body was found to be pink in color, in Papanicolaou stain, it was blue, and in acridine orange, it was fluorescent green in color. Interobserver blinding was done.

Results

The results were obtained using the Chi-square test for comparing the two groups and one-way ANOVA analysis was done for comparing the three methods within the same group.

The overall association between sex and Barr bodies revealed 73.33% positive results in females and 6.67% in males with 93.33% and 26.67% negative results, respectively, in males and females.

Overall, the statistical analysis of gender determination between male and female shows $P = 0.0004$ which was highly significant. Therefore, it is identified that females show 100% positivity.

Based on the henna and hibiscus criteria with interobserver assessment, we have given the score for each slide and calculated the mean and standard deviation for each stain for both nuclear morphology and crispiness of the stain using the one-way ANOVA test. It showed that Papanicolaou stain has better nuclear morphology and crispiness of the stain than Feulgen stain and acridine orange.

Discussion

In the present study, we differentiate the gender of an individual by the identification of Barr bodies in deciduous dental pulp. The association between sex and Barr bodies in deciduous teeth showed 85.71% negative result in males and 100% positive results in females. The overall association between sex and Barr bodies revealed 73.33% positive results in females and 6.67% in males with 93.33% and 26.67% negative results, respectively, in males and females.

The most significant association was observed between the type of teeth and Barr bodies. Here, deciduous teeth showed 60% positive results and 20% negative results.

Sex determination can be done by karyotyping, polymerase chain reaction (PCR), fluorescent body (Y chromatin) demonstration in male somatic cells, Davidson body in female neutrophils, and Barr body in female somatic cells.[5] Barr bodies are chromatin material which is present in the nucleus

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of female somatic cells and plays a pivotal role in gender identification in individuals.[6]

Ravishankar *et al*. reported that in females, the mean of Barr bodies was found to be 13 ± 2, and in the males, it was found to be 5 ± 3. Pulp tissues with a maximum number of Barr bodies belong to a female and pulp with a minimum number of Barr bodies belongs to a male.[7]

Nayar *et al*. used four groups for sex determination was done with PCR method which showed 100% accuracy in Group I, 86% accuracy in Group II, 73% in Group III, and 86% in Group IV.[8] The deciduous teeth contain immature mesenchymal stem cells, it is used to observe the earliest stages of gender determination in humans. The formation of stem-cell niche in dental pulp of deciduous teeth starts before birth and is maintained before eruption of the permanent teeth. This short period of an active growing of the organism is characterized by the maintenance of active stem-cell niche that is rich in stem cells, which are not heavily affected by accumulative effect of genetic and/or environmental factors.[9]

The present study involved deciduous dental pulp by staining them using Feulgen stain, acridine orange, and Papanicolaou stain and the result was more reliable in female deciduous pulp. By comparing the three stains based on the nuclear morphology and the crispiness of the staining, the result showed that Papanicolaou stain [Figure 1] was better when compared with other two stains. The sample size is small, increase in sample size will provide more accuracy.

The nuclear stains used in this study are to compare the feasibility and reliability of this procedure in chairside dental practice. Once the authenticity is proved with large sample size, it can be used in crime scenes and during mass disasters to identify the gender determination of the victim using a simple, cost-effective, and reliable technique.

Conclusion

Forensic investigations play an important role in the identification of the person. Sex determination gains first priorities in the process of identification of a person by a forensic investigator in the case of mishaps, chemical and nuclear bomb explosions, natural disasters crime investigations, and ethnic studies.[10] Dental tissue is resistant in nature to different environmental assaults, such as incineration and mutilation dental tissues offer an excellent source in forensic analysis. The dental pulp is encased in a sturdy shell of hard tissues of the teeth and is easily retrievable.[11] Hence, the gender determination of the person can be detected using dental pulp and staining with the Papanicolaou stain.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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