Original Article

Determination of Barr bodies in Transgender Patients in India – A comparative study

Aishwarya Lakshmi, Amritha James, Rameshkumar Annasamy, Rajkumar Krishnan

Department of Oral Pathology and Microbiology, SRM Dental College, Chennai, Tamil Nadu, India

Abstract

Sex determination in forensic medicine is considered one of the first and foremost steps in personal identification. The need for identifying the exact sex of the individual arises when deciding whether a person can exercise certain civil rights reserved for one particular sex, for competing in sex-specific athletic and sports events, legitimacy, divorce, paternity disputes and also to some criminal offenses. Nuclear sexing by Barr body examination can be done using buccal smears to establish the sex of the individual when routine methods fail to disclose the exact gender of the individual.

Aim: To determine and compare the Barr bodies present in exfoliated buccal epithelial cells in males, females and transgender populations using light and fluorescence microscopy.

Materials and Methods: A total of 90 patients were recruited for the study. Group I consisted of 30 female patients. Group II consisted of 30 male patients and group III consisted of 30 transgender patients. The buccal mucosa was then scraped using a wooden spatula and the cells obtained were fixed in 95% ethanol. Two smears per individual were made and stained. One smear was stained with papanicolaou (PAP) stain and the other with Acridine orange and viewed under light microscopy and fluorescent microscopy, respectively.

Results: When PAP stained slides were examined, the percentage of Barr-bodies in females ranged from 3% to 5% and in males it was 0% and in transgenders, it ranged from 0% to 5%. In Acridine orange stained smears, the percentage of Barr bodies in females ranged from 1% to 3% and in males it was 0% and in transgenders, it was 0%. Kruskal–Wallis test to study the relation of Barr body percentage in females, males and transgender subjects demonstrated significant differences between the groups ($P < 0.001$). Wilcoxon signed rank test was done for pairwise comparison, which showed that the distribution of percentage of positive cells in females are statistically significant from males and transgenders ($P < 0.001$).

Conclusion: Nuclear sexing using Barr bodies offers a simple yet effective method for determining the sex of transgender patients which could help them in understanding their gender identity better and diagnose any underlying chromosomal aberration.

Keywords: Acridine orange, Barr bodies, buccal smear, PAP, transgender

Address for correspondence: Dr. Amritha James, Department of Oral Pathology, SRM Dental College, Bharathi Salai, Ramapuram, Chennai - 600 089, Tamil Nadu, India.
E-mail: amrithajames94@gmail.com

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INTRODUCTION

Establishing individuality is imperative in any investigative procedure. Sex determination in forensic medicine is considered one of the first and foremost steps in personal identification. Often, determination of the sex of an individual becomes necessary in situations such as criminal cases and mass disasters where the individual's body is in an advanced state of decay and where primary sex organs are lost due to decomposition.[1]

Establishing individuality also becomes essential when the individual of one sex carries the features of the opposite sex or when a person appears to possess the primary sex organs of both sexes. Such need for identifying the exact sex of the individual arises when deciding whether a person can exercise certain civil rights reserved for one sex only, for competing in sex-specific athletic and sports events, legitimacy, divorce, paternity disputes and also to some criminal offences.[2,3]

Nuclear sex can be determined in various ways including Karyotyping, the study of the Fluorescent body (Y chromatin) and the examination of Barr bodies (X-chromatin). Barr bodies are known to arise from the inactivation of the X chromosome in a female cell. The study of Barr bodies is advantageous in that it can be studied even under an ordinary compound microscope with simple staining techniques. Barr bodies are Feulgen-positive, heteropyknotic and basophilic, intranuclear structures, seen in mammalian cells during interphase. Since they are nuclear structures and all nuclear structures are known to fluoresce, Barr bodies also fluoresce. Most often, they are noticed as densely stained condensed chromatin masses adjacent to the nuclear membrane.[4]

When conventional methods of sex determination fail, buccal smears could help in detecting the sex and thereby establish the identity of the individual. Nuclear sexing by Barr body examination can be done using buccal smears to establish the sex of the individual.[5] Hence the current study aims to determine and compare the Barr bodies present in exfoliated buccal epithelial cells in males, females and transgender populations using light and fluorescence microscopy.

MATERIALS AND METHODS

The study was conducted in the outpatient department after obtaining approval and ethical clearance from the Institutional Review Board (IRB APPROVAL NUMBER: IRB/2014/MDS). Male and female subjects were recruited from the outpatient department of SRM Dental College. The study groups composed of transgenders were recruited from the Sagodharan association. The study was conducted for a period of one year. Informed consent was obtained in writing from all the participants.

Sample size

Sample size estimation was done using G*Power 2® software. The power of the study was set at 80%, and the alpha error was set at 5%. Simple random sampling was done to recruit participants for the study. Based on the sample size assessed and the sampling technique used, a total of 90 patients were finally recruited for the study.

Inclusion and exclusion criteria

A total of 90 patients were recruited for the study. Group I consisted of 30 female patients. Group II consisted of 30 male patients and group III consisted of 30 transgender patients. Healthy patients between 21 and 40 years were recruited in all three groups. People with systemic diseases and deleterious oral habits were excluded from the study in all three groups.

For patients in group III, the organization did not have any medical records documenting their chromosomal and gender investigation status. Hence, their self-declared gender identity was considered to be the final word in their inclusion in to group III.

All the transgenders were male to female as self-declared (based on the self-identification model) and were projecting themselves as females by way of dressing and behaviour. Five of them were transsexuals (post Sex Reassignment Surgeries) and the remaining were either in pre-operative stage or non-operative transgenders (decision to not undergo any surgical correction). All 30 individuals had undergone hormonal therapy for feminising their features.

Sample collection

Before collection of the samples, subjects were asked to rinse their mouth with water. The buccal mucosa was then scraped using a wooden spatula repeatedly and gently while maintaining firm pressure to collect the buccal cells. The collected scraping was then transferred onto two clean glass slides.

Two smears per individual were made. Rapid fixation was done in 95% ethyl alcohol and stained. One smear was stained with PAP stain and the other with Acridine orange. After mounting with a cover slip, the PAP-stained slides were viewed under 100x oil immersion using a light

microscope and the acridine orange slides were viewed under 100x oil immersion using a fluorescence microscope.

Screening of slides

The stained and mounted slides were studied systematically. 50 cells per slide were counted. During the screening, to be identified as a Barr body, it had to be attached to the nuclear membrane or found freely in the nucleoplasm, appearing circular, disk-shaped or triangular in shape. Two observers examined the slides independently.

Statistical analysis

SPSS version 20 was used to analyse the data. Since the data did not follow the normal distribution, a non-parametric test was used for comparison between the groups. Comparison of the percentage of positive cells between groups was done using the Kruskal–Wallis test. Bonferroni corrected Wilcoxon signed rank test was done for pair-wise comparison. Inter-observer variability was assessed using Kappa statistics.

RESULTS

When PAP-stained slides were examined, the percentage of Barr-bodies in females ranged from 3% to 5% [Figure 1] and in males it was 0% and in transgenders, it ranged from 0% to 5% [Figure 2]. In Acridine orange stained smears, the percentage of Barr bodies in females ranged from 1% to 3% [Figure 3] and in males it was 0% and in transgenders, it was 0%.

The Kruskal–Wallis test was performed to study the relation of Barr body percentage in females, males and transgender subjects which demonstrated significant differences between the groups ($P < 0.001$) [Table 1].

Bonferroni corrected Wilcoxon signed rank test was done for pairwise comparison which showed that the distribution of the percentage of positive cells between males and transgenders was not statistically significant ($P > 0.05$) whereas the distribution of the percentage of positive cells in females are statistically significant from males and transgenders ($P < 0.001$) [Tables 2 and 3].

Table 1: Kruskal–Wallis to compare percentage of positive cells per slide between Groups

	Group			
	Female	Male	Transgender	*P*
Positive cells – PAP Stain				
n	30	30	30	<0.001
Mean	4.2	0.0	0.4	
Std. Dev	0.9	0.0	0.4	
1st quartile	3.0	0.0	0.0	
Median	4.0	0.0	0.0	
3rd quartile	5.0	0.0	0.0	
Positive cells – Acridine orange				
n	30	30	30	<0.001
Mean	2.3	0.0	0.0	
Std. Dev	0.9	0.0	0.0	
1st quartile	1.0	0.0	0.0	
Median	3.0	0.0	0.0	
3rd quartile	3.0	0.0	0.0	

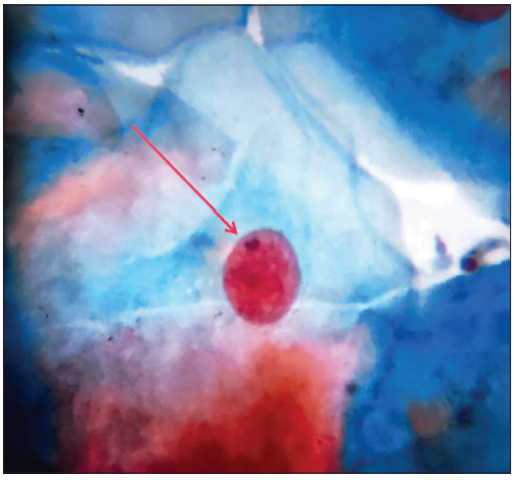

Figure 1: Presence of Barr body lying close to the nuclear membrane; Female Patient – PAP stain 100x

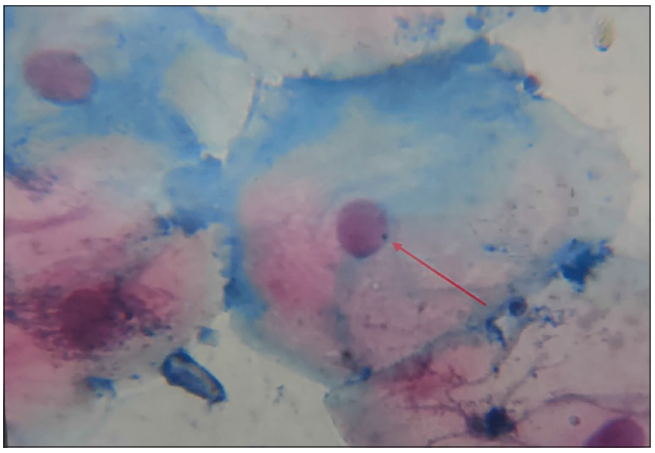

Figure 2: Presence of Barr body lying close to the nuclear membrane; Transgender Patient – PAP stain 100x

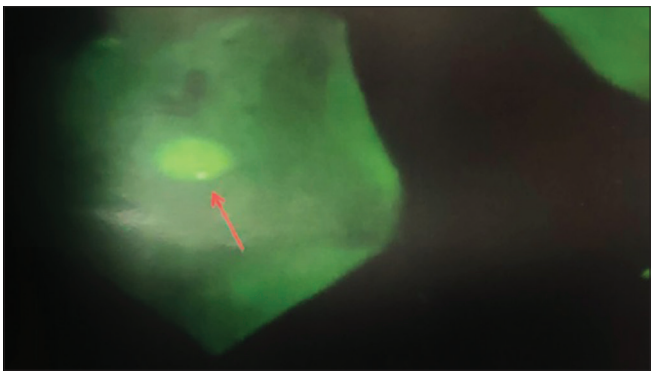

Figure 3: Presence of Barr body lying attached to the nuclear membrane; Female patient – Acridine orange 100x

Comparison between the proportion of Barr body-positive cells in PAP stain and acridine orange staining revealed that the PAP stain was superior in identifying the presence of Barr bodies. It was statistically significant between PAP and Acridine orange stain ($P < 0.001$) [Tables 4 and 5]. Inter-observer agreement is highly significant in identifying the presence of Barr body in each group ($P < 0.001$) using kappa statistics.

DISCUSSION

Sex determination is an important aspect of personal identification. Barr bodies are known to arise from the inactivation of the X chromosome in a female cell. This process of inactivation is known as lyonization. The study of sex chromatin provides sufficient evidence for the determination of the sex of the individual and has been studied widely in sex determination. Sex chromatin has been studied in buccal epithelial cells, fibroblasts of Pulp, cervical cells, skin and hair.[6,7]

The presence of Barr body in females represents them as chromatin positive, while the absence of the Barr body in males represents them as chromatin negative. This characterisation of Barr bodies offers a way to identify the true, underlying sex in those who are sexually anomalous or intersexed individuals.[8]

Several studies have emphasized the role of Barr bodies in sex identification, however, our study is unique in that it was done to determine the number of Barr bodies in transgenders patients.[5,9,10] The term transgender is an "umbrella" term that is used to describe a wide range of identities and experiences, including but not limited to pre-operative, post-operative and non-operative transsexual people; male and female cross-dressers (sometimes referred to as "transvestites"); intersexed individuals; and men and women, regardless of sexual orientation, whose appearance or characteristics are perceived to be gender atypical.

Our study comprised of three groups: Group I included 30 female subjects out of which all the 30 samples showed the presence of Barr bodies in both PAP and Acridine orange stain and the percentage of Barr bodies ranged from 3% to 5% under PAP stain and 1% to 3% under Acridine orange. Group II comprised of 30 male subjects out of which all 30 samples showed the absence of Barr bodies in both PAP and Acridine orange stain and the percentage of Barr bodies was estimated at 0%. The percentage of Barr body-positive cells was less in our study when compared to other studies.

Table 2: Bonferroni adjusted Wilcoxon test for pair-wise comparison

Variable	*P*		
	Female vs Male	Female vs Transgender	Male vs Transgender
Positive cells – PAP Stain	<0.001	<0.001	0.999
Positive cells – Acridine orange	<0.001	<0.001	0.99

Table 3: Wilcoxon Signed Ranks Test to compare percentage of positive cells per slide between PAP Stain and Acridine Orange methods in each gender separately

Group	*P*
Female	<0.001
Male	1.000
Transgender	0.102

Table 4: Cross table to compare proportions between Barr bodies and Groups

	Barr bodies PAP Stain			
	Absent		Present	
	n	%	*n*	%
Group				
Female	0	0	30	100
Male	30	100	0	0
Transgender	27	90	3	10
Total	57	63.3	33	36.7

Table 5: Cross table to compare proportions between Barr bodies and Groups

	Barr bodies Acridine Orange			
	Absent		Present	
	n	%	*n*	%
Group				
Female	0	0	30	100
Male	30	100	0	0
Transgender	30	100	0	0
Total	60	66.7	30	33.3

The results of our study were in concordance with the study by Walter Hermann *et al.*, who studied the percentage of Barr bodies in oral smears in 100 subjects (50 male and 50 female) using PAP stain. He reported that all the females were positive for Barr body with a percentage ranging from 10% to 32% and all males were negative with a percentage of 0%.[5] Suazo *et al.* in their study, observed sex chromatin in cells of the pulp tissue with 20 samples from male and female subjects. Barr bodies were present in all 20 female samples with 20.4% Barr body-positive cells. There were no positive cells in male subjects. So, they concluded that dental pulp is a reliable test for sex determination.[10] In our study, the distribution of the percentage of Barr body-positive cells in female is statistically significant from the male ($P < 0.001$).

In our study, the percentage of Barr body-positive cells varied with PAP and acridine orange stain. PAP stain

showed a slightly higher percentage of Barr body-positive cells when compared with Acridine Orange staining. This was however in contrast to other studies. Datar U *et al.* in their research, studied the presence of Barr bodies in buccal smears using PAP and Aceto orcein and concluded that Aceto Orcein was a better stain than PAP for visualizing nuclear details.[11] Reddy *et al.* in their study to assess Barr bodies in the exfoliative cells using Acridine orange staining, concluded that a higher percentage of Barr body-positive cells was demonstrated in the female group using Acridine orange stain.[12]

In our study, Group III, comprised of 30 Transgender subjects and only 3 subjects showed the presence of Barr body with a percentage of positive cells ranging from 1% to 3% and the remaining 27 subjects showed the absence of Barr body under PAP stain. Under Acridine orange all the 30 subjects showed the absence of Barr bodies.

The 27 subjects who were chromatin negative could have been born as males but with a gender orientation wanting to project themselves as females. And among these individuals, although a majority of them may be normal XY males with opposite gender orientation and identification, it is possible that they may also include individuals, having chromosomal aberrations like Complete/incomplete Androgen insensitivity syndrome with XY nuclear chromatin or True hermaphrodite with predominant XY and female gender identity.[13,14]

The 3 subjects who were found to be chromatin positive could have chromosomal aberrations like 47 XXY with female gender identity, true hermaphrodite with predominant XX and a female gender or 46XX with CAH, 45 XO/46 XX mosaicism.[15,16]

Limitations and future considerations

The current study is not without limitations. The sample population was relatively small. The scope of this study did not permit karyotyping and hence any chromosomal aberration if present couldn't be assessed. The biology of gender is far more complicated than XX or XY chromosome. It is remotely possible that self-identified transgenders may be presenting with other known anomalies that will definitely require medical evaluation and intervention apart from routine hormonal and surgical correction. These findings need to be studied and validated on a larger sample size using PCR and karyotyping for a better understanding of the sexual identity of the transgender population.

CONCLUSION

The current study was done to determine and compare the Barr bodies present in exfoliated buccal epithelial cells among the different genders. Though the exact essence of gender identity can only be assessed by karyotyping, nuclear sexing using Barr bodies offers a simple yet effective method for determining the sex of transgender patients which could help them in understanding their gender identity better and diagnose any underlying chromosomal aberration.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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