

Original Research Article

Buccal Barr Bodies: Accuracy and Reliability in Sex DeterminationNavdeep Kaur¹, Ruhi Sidhu², Sunira Chandra³, Neeraj Taneja⁴¹Senior Lecturer, Department of Oral Medicine & Radiology, Rayat & Bahra Dental College & Hospital, Village-Sahauran, Mohali, Punjab, India²Senior Lecturer, Department of Oral Medicine & Radiology, Laxmi Bai Dental College & Hospital, Patiala, Punjab, India³Reader, Department of Oral Medicine & Radiology, Saraswati dental college & Hospital, Lucknow, Uttar Pradesh, India⁴Professor and Head, Department of Oral Medicine & Radiology, Rayat & Bahra Dental College & Hospital, Village-Sahauran, Mohali, Punjab, India***Corresponding Author:**

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Abstract: Forensic dental fieldwork requires an interdisciplinary knowledge of dental science. Barr bodies are known to arise from inactivation of x- chromosome in a female cell, so it also has been used as a reliable method for sex determination. The objective of this study is to evaluate the reliability and accuracy Buccal Barr bodies for sex determination. The study was conducted on 100 subjects, (50 males and 50 females) with an age range of 20 to 36 years. The method was applied on each subject and the data was collected. After obtaining the data it was coded, analysed, decoded, interpreted and statistically analysed. The sensitivity and specificity of buccal barr bodies was found to be 24% and 84% respectively with an accuracy of 54%. It is concluded that buccal Barr bodies have the accuracy (54%). Hence it can be one of the reliable parameter but more studies are required to justify role in forensic odontology for sex determination, but certain circumstances like delay in sample staining, storage incapability can alter the results. Hence, more samples with accurate sample collection and storage technique can provide better results.

Keywords: Forensic Science, Buccal Barr bodies, Barr body, Sex determination, Human identification, Personal identification

INTRODUCTION

Establishing individuality is an imperative aspect in any investigating procedure. There are numerous means and ways to do so in human beings (either alive or dead) when a human body is in its entirety but very few when only part(s) is available. Human specimens, such as blood, semen, hair, and saliva stains containing buccal mucosal cells, found at the scene of crime or on a lethal weapon, are of major help in solving criminal cases. In cases of sexual offences, the buccal mucosal cells along with saliva stains are found in various parts of the body and also at the scene of crime [1].

The sex chromatin or Barr body is a condensation of chromatin present at the nucleus of cells in female individuals. Their observation is possible in different cell types and is used for the rapid diagnosis of biological sex. In 1949, Dr Murray L. Barr, a Canadian cytogeneticist was the first one to described the phenomenon that in mammals, males are

heterogametic (XY) and females homogametic (XX). Dosage compensation is achieved by inactivation at random of one of the two X chromosomes. The hetero chromatinized X chromosome appears as darkly-staining bodies attached to the nuclear membrane [2].

Later, in 1950 Barr and Bertram determined that there was a difference between male and female cells. They found that, in female cells, there was a small chromatin condensation at the nuclei of nerve cells of cats.³ In 1956 Dixon & Torr found sex chromatin (Barr Bodies) in oral mucosal cells [3].

Barr body testing was introduced in the 1966 Olympic games, in an effort to detect male athletes trying to "pass" as females, to gain a competitive advantage. Teams from Eastern Europe were particularly suspected. Such allegations had been made for many years, and a number of athletes were stripped of their medals as a result of ambiguous genital sex [4].

In 1961, Lyon outlined the X-inactivation or what is commonly known as the Lyon hypothesis [1]. It states

- (1) Only one of the X chromosomes is genetically active.
- (2) The other X of either maternal or paternal origin undergoes heteropyknosis and is rendered inactive.
- (3) Inactivation of either the maternal or paternal X occurs at random among all the cells of the blastocyst on or about the 16th day of embryonic life.
- (4) Inactivation of the same X chromosome persists in all the cells derived from each precursor cell.

Thus, the great preponderance of normal women are in reality mosaics and have two populations of cells, one with an inactivated maternal X and the other with an inactivated paternal X.

Barr bodies are feulgen positive, heteropyknotic, 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. In some cells (especially neurons), they can be observed adjacent to the nucleolus or even free in the nucleoplasm. They can be plano-convex, biconvex, triangular, spherical, or rectangular in shape when observed under ordinary microscope in oil immersion or high power. Sometimes, they resemble the letter V, W, S, or X under electron microscope. They measure about 0.8 to 1.1 μm in diameter [1] (Figure 1).

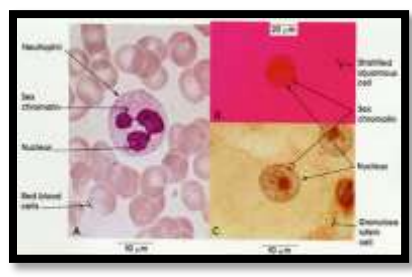


Fig-1: Barr Body

The present study was conducted to evaluate the reliability and accuracy of Buccal Barr bodies for the sex determination and to justify its role in forensic dentistry.

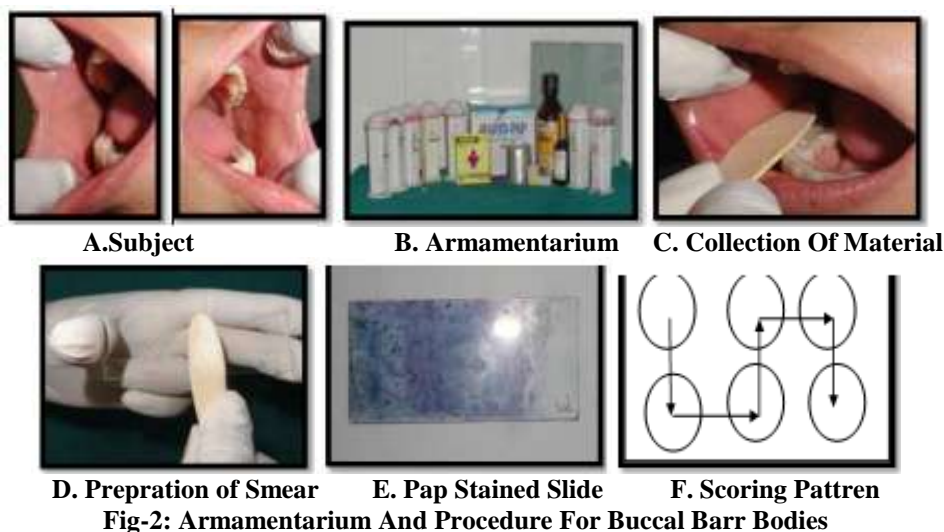
MATERIAL AND METHODS

The Study was carried out in Department of Oral Medicine and Radiology, Teerthanker Mahaveer Dental College and Research Center, Moradabad, Uttar Pradesh after the required approval from the ethical committee. Study included 100 subjects, including 50 males and 50 females of age between 20 to 36 years above. A thorough detailed oral examination was conducted and the data was collected as per Performa. After explaining the procedure to the patient the written consent was obtained. Only those patients were selected who were systemically healthy.

The data was collected and coded. After analysis, the data was decoded, interpreted and statistically analysed. Selected subjects were asked to rinse the mouth with Chlorhexidine mouth wash and then with water. A sterilized wooden spatula was drawn along the buccal surface of the cheek [Figure 2 (c)]. The cellular material was quickly smeared on the slide and was fixed immediately with 90% ethyl alcohol for 15-30 minutes [Figure 2 (d)].

Papanicolaou Staining: The fixed smear was dipped in tap water for a minute and excess water was blot out. Slide was dipped in PAP nuclear stain for a minute and was washed in Scotte's tap water buffer for 30 seconds followed by blot out of excess water from slide. Then the slide was dipped in Rapid PAP Dehydrant number 1 and then in number 2 for 30 seconds each. Slide was dipped into working cytoplasmic stain for 45 seconds followed by thorough washing in scotte's tap water buffer. Dehydration was repeated in second bath of Rapid PAP Dehydrant for 30 seconds. Slide was kept for air drying. After complete drying, slide was dipped in xylene to remove excessive stain and was mounted with cover slip using a drop of DPX mount [Figure 2 (e)].

Scoring: The slides were observed under the supervision of oral pathologist under a binocular microscope at 40X magnification. A total of 100 cells were scored for the presence of Barr bodies in a zigzag manner at 40X magnification and their presence was confirmed under 100 X magnification (oil immersion) [Figure 2 (f)]. Photomicrography- Photomicrographs were obtained for the BMCyt assay parameters using Olympus E-420 camera fitted on Olympus microscope.



As Barr bodies are densely stained condensed chromatin masses adjacent to the nuclear membrane. They can be plano-convex, biconvex, triangular, spherical, or rectangular in shape when observed under ordinary microscope in oil immersion or high power under electron microscope they may resemble letter V,W,S or X , with diameter of 0.8-1.1um (Figure 1)

The data collected from the subjects were interpreted and statistically analyzed using software SPSS version 19.0. Chi square and discriminant functional analysis test was done.

RESULTS AND OBSERVATIONS

Table 1 and fig 3 shows that out of 50 males Barr bodies were absent in 42 subjects (84%) and were present in 8 subjects (16%). In 50 females Barr bodies were absent in 38 subjects (76%) and were present in 12 subjects (24%). In 100 subjects Barr bodies were absent in 80 subjects (80%) and present in 20 subjects (20%). The probability of chi-square statistic 1.0 at $df = 1$ was found to be 0.317 which was larger than the alpha level 0.05. Hence the null hypothesis of no difference was accepted. This does not support the research hypothesis that there is significant difference between the presence of Barr bodies in male and female subjects

Table-1: Showing Discriminant functional analysis

Classification Results ^a				
Sex		Predicted Group Membership		Total
		Male	Female	
Count	Male	32	18	50
	Female	12	38	50
Percentage (%)	Male	64.0	36.0	100.0
	Female	24.0	76.0	100.0
70.0% of original grouped cases correctly classified				

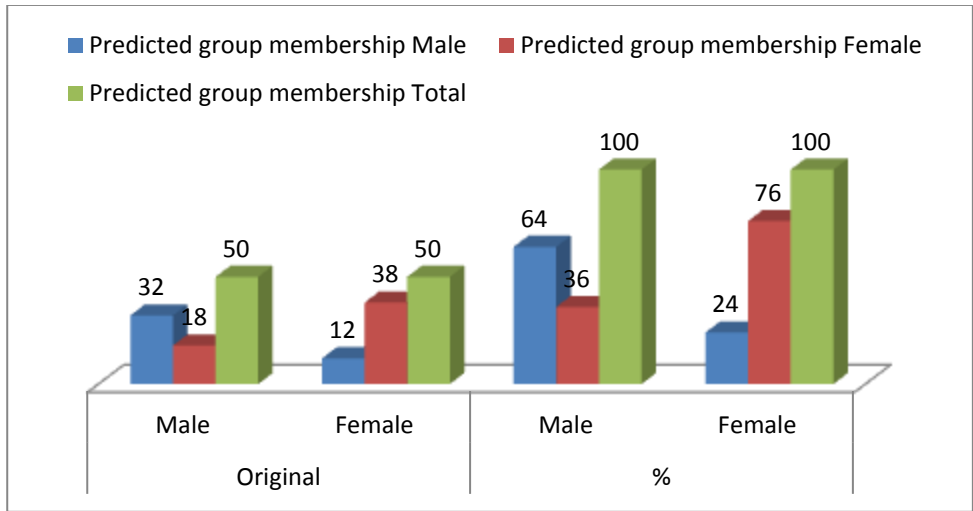


Fig-3: Showing Classification results of cheiloscopy

Table 2 and Fig 4 shows the discriminant functional analysis of the actual group membership against the predicted group membership, in which out

of 50 males 42 (84%) were correctly classified and 50 females 12 (24%) were correctly classified. Hence, 54% of original grouped cases were correctly classified.

Table-2: Showing frequency cross-tabulation of gender wise distribution of Barr body

Sex * Barr Bodies Crosstabulation					
			Barr Bodies		Total
			Absent	Present	
Sex	Male	Count	42	8	50
		% within Sex	84.0%	16.0%	100%
	Female	Count	38	12	50
		% within Sex	76.0%	24.0%	100%
Total		Count	80	20	100
		% within Sex	80.0%	20.0%	100%

Chi-square= 1.000 df=1 p=0.317 Non-significant

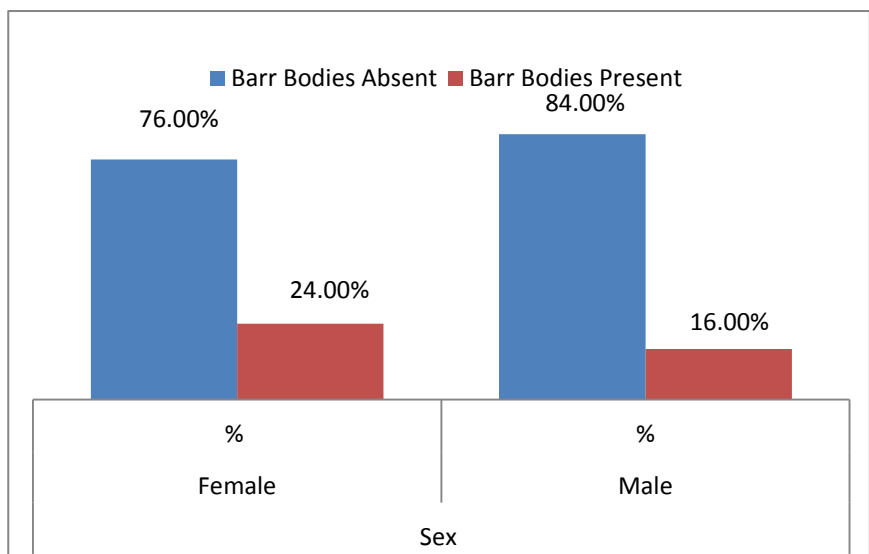


Fig-4: Showing Frequency of gender wise distribution of Barr Bodies

DISCUSSION

Demonstration of nuclear sex plays a vital role as far as sexing of the individual is concerned. The sex chromatin or Barr body is a condensation of chromatin present at the nucleus of cells in female individuals. Their observation is possible in different cell types and is used for the rapid diagnosis of biological sex. Subjects comprised of 100 subjects (50 males and 50 females). Similar studies were conducted by A. D. Dixon and J. B. D. Torr [5] on 260 individuals (126 males and 98 females), Vincent E. Aimakhu and A.I. Kadiri [6] on 405 babies (205 males and 200 females), Claudette Hajaj Gonzalez *et al.* [7] on 38 babies (19 males and 19 females), Nirmal Das *et al.* [8] on 100 individuals (50 males and 50 females). Mittal *et al.* [1] on 200 subjects (100 males and 100 females).

In our study age of the subjects ranged from 20 years to 35 years for males and 20 years to 27 years in females with the mean age of 23.62 years for males and 21.84 years for females. Similar studies were conducted by A. D. Dixon and J. B. D. Torr [5] who conducted a study on sample of age ranging between 16 months to 60 years, David W. Smith *et al.* [9] Vincent E. Aimakhu and A.I. Kadiri [6] Claudette Hajaj Gonzalez *et al.* [7] conducted a study on new born babies, Iván Suazo Galdames *et al.* [10] conducted a study on sample between 14 to 44 years.

In our study buccal mucosal scraps were obtained with a wooden spatula followed by smear preparation using Papanicolaou stain for detection of Barr bodies. However, samples can also be collected from hair root sheath, blood, bone, pulpal tissue, buccal smears, saliva, and semen [5]. In the present study we opted for buccal smears because buccal epithelial cells can be collected easily with simple, inexpensive, quick and in non invasive manner, also not much of the studies have been conducted using buccal smear. PAP stain smears were than analyzed by Zigzag method to view all the slides. Similar study was conducted by Mittal *et al* [1] A. D. Dixon and J. B. D. Torr [5] and David W. Smith *et al.* [9] who collected buccal smear for identification of Barr bodies. Whereas Nirmal Das *et al.* [8] conducted a study on pulpal tissue and Patricia Munoz *et al.* [11] on exhumed Bones. Vincent E. Aimakhu and A.I. Kadiri [6] in his study used Giemsa stain to view barr bodies. Iván Suazo Galdames [3] used hematoxylin eosin stain and they analyzed barr bodies using Micrometrics SE Premium software. A. D. Dixon and J. B. D. Torr [5] used 1% cresyl violet to view barr bodies.

Our study showed 24% presence of barr body in females and 16% presence of barr bodies in males. Which was statistically insignificant with p value 0.317(Table 1). Reduce number or presence and absence of barr body in females depends on various factors like menstruation cycle, pregnancy, Turner's

syndrome and ovarian dysgenesis which shows male sex chromatin pattern, and in males sexual problems, patients with Trisomy X and Klinefelter's syndrome which shows female sex chromatin pattern. Use of different stains can also lead to variations like in the present study we found 16% presence of Barr body in males this can be due to the use of non specific stain (PAP). Use of PAP stain can sometimes make Barr bodies, RNA material, debris, look alike and which makes it difficult to differentiate. Also method of sample collection that is scraping of the buccal mucosa can damage the cell and can show absence of the barr bodies [12].

Results was in accordance of studies present in literature by Nirmal Das *et al* [8] in which it showed that maximum number of Barr body was found to be 8 in females and 6 in male. He concluded that number of Barr bodies decrease in 15 – 21 days study period at optimum temperature of 28.6 °C to 35.6 °C. This could be the reason for reduced number of Barr body among female sample. The same delay happened with some of the samples of our study which to some extent justify our result. Study conducted by A. D. Dixon and J. B. D. Torr [5] David W. Smith *et al.* [9] Vincent E. Aimakhu and A.I. Kadiri [6] Claudette Hajaj Gonzalez *et al.* [7] Patricia Munoz *et al.* [11] showed absence of Barr bodies in males.

The sensitivity and specificity of this parameter was found to be 24% and 84% respectively with an accuracy of 54% (Table 2). Literature search revealed no study has provided sensitivity, specificity.

CONCLUSION

The sex chromatin or Barr body is a condensation of chromatin present at the nucleus of cells in female individuals. Their observation is possible in different cell types and is used for the rapid diagnosis of biological sex. The sensitivity and specificity of this parameter was found to be 24% and 84% respectively with an accuracy of 54%. Literature search revealed no study has provided sensitivity, specificity. The present study concluded that Barr Body estimation through buccal mucosal scraps can be a reliable method for sex determination, but certain circumstances like delay in sample staining, storage incapability can alter the results. Hence, more samples with accurate sample collection and storage technique can provide better results.

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