

# Special Stains to Disclose Barr Bodies in Buccal Scrape for Gender Determination in Forensic Science

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## ABSTRACT

**Introduction:** Sex determination holds a valuable significance in these circumstances to clear medico-legal problems. In some instances the sample available might be very minute, in the form of only few cells which can be used to disclose the gender and identity of a person. One of the simplest methods of sex determination is visualizing Barr bodies from cytosmears of buccal mucosa using special stains. Study aimed to assess the easiest staining method for quick detection of Barr bodies to determine the sex of an individual using epithelial cells from buccal scrapes.

**Material and methods:** A total of 50 individuals, 25 males and 25 females were considered for the study. Buccal scrapes and peripheral blood smears were obtained from each patient. Peripheral smears would be stained with Leishman's stain and used as a standard whereas the cytosmears from buccal scrapes would be assessed for Barr bodies using routine hematoxylin and eosin stains and special stain alkaline methylene blue.

**Results:** In females peripheral smears showed 100% positivity for Barr bodies whereas 88% cases were positive for special stain alkaline methylene blue and 80% cases showed positivity for hematoxylin and eosin stain.

**Conclusion:** Alkaline methylene blue can be used as an easiest and quickest staining technique for sex identification.

**Keywords:** Forensic, Gender determination, Buccal Scrapes, Alkaline Methylene Blue, Barr Bodies

(of the tooth, skull and other soft tissues of oral and paraoral region) or by using molecular analysis<sup>5,6</sup> which involves conventional technique like Polymerase Chain Reaction (PCR) and karyotyping which are expensive and time consuming.<sup>7</sup>

In the head and neck region amongst various sources like tooth, blood, hair and saliva used for identification, it has been proven that buccal mucosal scrapes forms the simplest, quick and easiest method of sex determination by identification of the female sex chromatin, the Barr bodies using staining techniques like Haematoxylin and Eosin(H and E) and Papanicolau(PAP) staining technique.<sup>8</sup>

## Sex identification using Barr body

The Barr bodies are present in 40% of females who are considered as chromatin positive and absent in males who are considered as chromatin negative.<sup>8</sup>

Presence of Barr bodies in oral smears was first studied in cats in the year 1940. They were found only in female and were first named by Barr and Bertem (1949).<sup>8</sup>

The significance of these bodies was first given by Lyon. He suggested that inactivation of one of the X chromosome in each somatic cell occurs during the early embryonic development and named such process as lyonization.<sup>9</sup>

Barr bodies are unique chromatin structures formed in nuclei of the mammalian females as a means of sex chromosome dosage compensation.

Barr body are present adjacent to the nuclear envelope in 75-80% of inter phase cells. Studies using Barr bodies from the pulp tissue of extracted teeth using regular H and E have shown 100% positivity in females and negativity in males.<sup>10</sup>

They generally appear as basophilic structures with varying morphology which can be either spherical, rectangular, plano-convex, biconvex, or triangular measuring  $0.8 \times 1.1$  microns.

Special stains on buccal scrapes for detection of Barr bodies for sex determination has been used as an important tool in forensic analysis as it provides 95-98% accuracy, making it a significant accessory for sex determination.<sup>11</sup>

PAP stain is the most commonly used staining technique which

## INTRODUCTION

In the modern era, society is faced with challenges in all conceivable area. Despite the advancement in modern technology, medical breakthroughs and the geographical changes, crime still persists in all walks of our lives.<sup>1</sup> Forensic odontology is emerging as a potential branch in dentistry and awareness of this speciality among the dental fraternity is unfortunately less in India compared to the rest of the world. It is the specialty with the goal of investigating psychological, physical, chemical and biological phenomena. It comprehends various aspects of human identification; criminal, civil, labor and administrative forensic investigation.<sup>2,3</sup> Human identification is one of the major fields of study and research in forensic science because it deals with the human body and aims at establishing human identity.<sup>3</sup> A great deal of effort is spent on the identity or confirmation of identity of the victim(s) and perpetrator(s).<sup>4</sup> Gender determination is a subdivision of Forensic Odontology and plays a very crucial role when information relating to the deceased is unavailable. Sex determination becomes the first priority 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.<sup>5</sup>

Sex determination can be done either by morphometric analysis

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very recently has been replaced by acridine orange for its specificity.<sup>11</sup>

The present study was carried out using Leishman's stain on peripheral smear, taken as standard; modified H and E stain and alkaline methylene blue on buccal scrapes with the aims and objectives to determine the presence of Barr bodies using special stain taking peripheral blood smear as standard and by using H and E and special stain (alkaline methylene blue) in buccal scrapes, to assess the percentage of Barr body positivity in female buccal scrapes and comparing them with peripheral smear and assessment of the easiest staining method for quick identification of sex through Barr bodies using epithelial cells in oro-facial region.

## MATERIAL AND METHODS

A total of 50 patients, 25 males and 25 females were considered for the study. A total of 100 buccal scrapes (2 scrapes from each patient) and 50 peripheral blood smears were taken. The patients who visited the Pathology Laboratory for routine investigation were considered for the study. The buccal scrapes were fixed in 95% formalin and stained using a conventional modified H and E stain and a special stain alkaline methylene blue. The peripheral smears were stained using Leishman's stain.

The modified H and E technique was followed to stain one of the scrapes from each patient. The scrapes were fixed in 95% alcohol. They were hydrated using decreasing grades of alcohol (100%, 90%, 80%) followed by water. The scrapes were then stained with Mayer's hematoxylin for 12 min, decolorized using 1% acid alcohol followed by blueing in tap water for 5 min. They were counter stained using eosin for 2 min and then dehydrated using increasing grades of alcohol. The scrapes were then cleared and mounted.

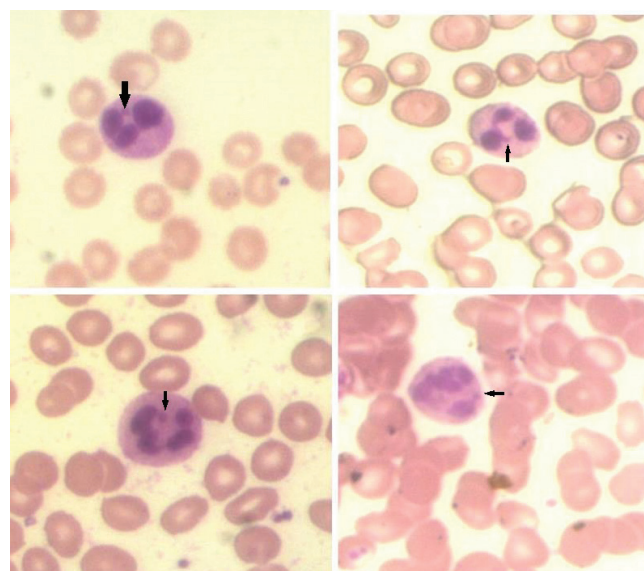
The other scrape was stained using alkaline methylene blue technique where a drop of water was taken on the slide. The patients were asked to scrape the buccal mucosa with the finger and mix it in the water droplet taken on the slide. The smear was spread using a slide and was not fixed. Alkaline Methylene blue was added drop by drop over the smear and left for 3 minutes and then was drained, washed and seen without mounting. Peripheral smears were stained using Leishman's stain where the blood smear spread on the glass slide was air dried. The smear was covered by undiluted Leishman's stain which was prevented from overflowing by adding drop by drop. A standard of 8-10 drops were added and left for 2 min 30sec. Twice the volume of buffered water (pH 6.8) was added, not allowing to overflow. It was left for 15 min. The stain was washed with clean water, air dried and observed under oil immersion.

## STATISTICAL ANALYSIS

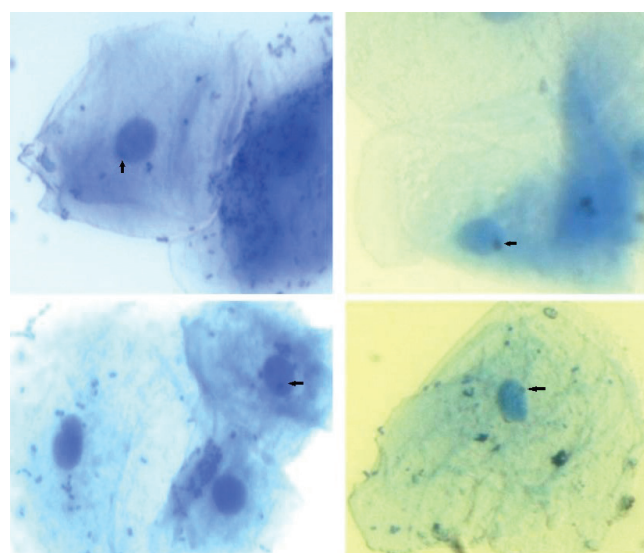
Descriptive statistics like mean and percentages were used to interpret the results. Microsoft office 2007 was used for the statistical analysis.

## RESULTS

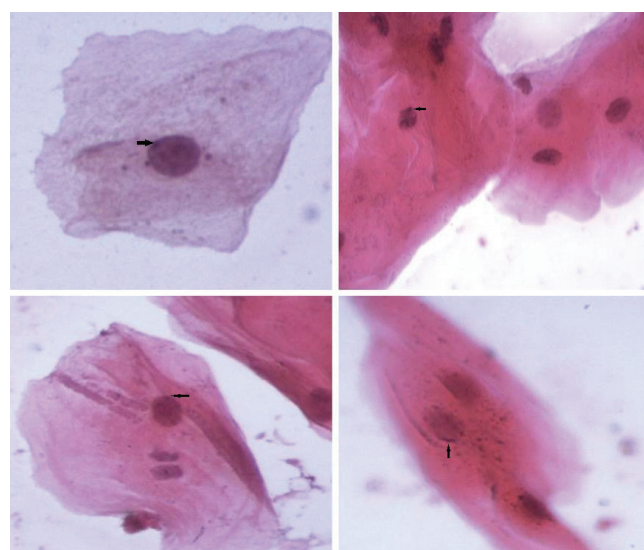
The number of patients and the detection of Barr bodies which appeared as dumbbell shaped extension from the lobed nucleus in neutrophils of peripheral smear as seen in Figure 1 and hyperchromatic dot in the periphery of the nucleus or in the nuclear membrane, in the buccal smear as seen in Figure 2 and 3 were tabulated as presented in table 1. The highest specificity



**Figure-1:** Appearance of Barr bodies in the peripheral blood smear using Leishman stain under 100x oil immersion



**Figure-2:** Appearance of barr bodies in buccal scrapes using alkaline blue special stain under 40x magnification



**Figure-3:** Appearance of barr bodies in buccal scrapes using hematoxylin and eosin stain under 40x magnification.

Number of patients	barr body in peripheral smear	barr body in bucal scrape: (H and E)	barr body in buccal scrape: (methylene blue)
25 females	++	+	+
25 males	--	--	--
++: High specificity of barr body detection; + : Low specificity of barr body detection.			

**Table-1:** Specificity of detection of Barr bodies with each technique.

Number of patients	Barr body positive	Barr body negative
25 females	++	none
25 males	none	25
++: high specificity of barr body detection		

**Table-2:** Peripheral Blood Smear: (Leishman Stain):

Number of patients	Barr body positive	Barr body negative
25 females	21	5
25 males	none	25

**Table-3:** Buccal Scrapes (H and E Routine Staining)

Number of patients	Barr body positive	Barr body negative
25 females	22	2
25 males	none	25

**Table-4:** Buccal Scrape: (Special Stain: Methylene Blue)

of detection was graded as ++ and the lowest specificity was graded as +. The detection of Barr body in peripheral smear using Leishman's stain was graded as presented in Table 2. The detection of Barr bodies in buccal scrapes using modified H and E stain is graded as presented in table 3 and using alkaline methylene blue is presented in table 4.

The peripheral blood smear showed 100% positivity for the Barr bodies. In females, about 88% of methylene blue stained buccal scrapes showed positivity for Barr bodies whereas 84% of buccal scrapes stained with H and E stain showed positivity. None of the peripheral smears and buccal scrapes showed positivity for Barr bodies in males.

## DISCUSSION

The most important challenge of the science of criminal investigation is the concrete identification of persons, subjects, scenes and actions, possibly connected to the crime. At the beginning, the bases for identification were served only by the testimonies of the witnesses and the traces visible to the naked eye, fixed at the scene. As a result of the revolutionary development of natural sciences, the circle of appropriate traces being brought under forensic examination has increased.

The principle of forensic identification resting upon practical experiment, has also stepped into a new dimension. The most significant base of forensic identification is the principle of individuality and relative durability. Sex determination becomes the first priority 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.<sup>12</sup>

Sex determination analysis can be done either by morphological analysis or by molecular analysis. Morphological analysis can be done on hard tissues (odontometric, orthometric and

miscellaneous) of oral and paraoral regions or soft tissue (lip prints-Cheiloscopy, palatal rugae pattern-Rugoscopy). Molecular analysis involves the study of DNA from extracted pulp, cartilage, hair, skin, buccal mucosa, epithelium attached to denture and toothbrush.<sup>5</sup>

Studies on buccal scrapes for identification of Barr body for gender determination were done using Papanicolaou stain, the most recent being a study by Mittal et al where 200 samples were obtained from buccal mucosa using metal spatula and smeared in the frosted slides followed by fixation in 95% ethyl alcohol for 10-15 min. Papanicolaou stain showed 40% positivity for Barr bodies in females.<sup>13</sup>

The short comings of the above technique was overcome by Reddy DPS et al who used acridine orange staining method using 100% alcohol as fixative for 15 minutes in a sample size of 40 of 20 each of males and females. The technique gave better contrast and accuracy as 72% of females showed positivity for Barr bodies as viewed in confocal imaging in comparison to PAP technique using compound microscope.<sup>14</sup>

The alkaline methylene blue staining technique showed concrete results in gender identification, in comparison with routinely practiced, most feasible H and E staining technique while taking Leishman stained peripheral blood smear that yielded 100% positivity as a standard as seen in our study.

Taking H and E staining method, which is the most feasible and routinely practiced technique in the field of pathology, in comparison with the peripheral blood smear which yielded 100% positivity for Barr bodies, alkaline methylene blue staining technique showed concrete results in gender identification as seen in our study.

Orofacial epithelial cells can play a very important role in sex identification, specifically the buccal epithelial cells by detecting the Barr body. In the present study, routine hematoxylin and eosin stain showed 84% positivity and alkaline methylene blue gave 88% positive results for detection of Barr bodies in females. Alkaline methylene blue being a very simple stain in preparation can be used as very quick and easiest method of sex identification in the field of crime or mass disaster as the smear of the subject needs no fixation and the procedure takes very less time (> 4 minutes) as compared to the PAP stain and acridine orange. The identification procedure can yield immediate results thereby simplifying further investigation.

The Barr bodies are very clearly evident as condensed dark violet or blue colour body in the nuclear membrane. The procedure was compared with peripheral smear in the same subjects chosen and along with the routine H and E stain. Both H and E and methylene blue stains showed complete negative results in the male counterpart.

## CONCLUSION

Both routine H and E staining procedure and alkaline methylene blue stains can aid in sex identification. Alkaline methylene

blue procedure is more simpler, economical, less technique sensitive and quicker as compared to earlier procedures used and more importantly, can be used at the site of accident. It can overcome the burden of using sophisticated techniques in mass disaster cases which can be expensive and time consuming. This procedure can be proven inceptive in forensic investigations specifically in conditions of very restricted and definite resources. The shortcoming of this technique could be the storage of these samples (slides) for future references as there is no fixative used.

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