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Original Research

Assessment of accuracy of Barr bodies in sex determination

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ABSTRACT:

Background: Establishing the gender of a person is of paramountimportance in forensic science. The present study was conducted to assess accuracy of Barr bodies in sex determination. **Materials & Methods:** 80subjects of both genders 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. Papanicolaou Staining was performed. 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). **Results:** Barr bodies were present in 15% males and 28% females and absent in 85% males and 62% females. **Conclusion:** Barr Body estimation through buccal mucosal scraps can be a reliable method for sex determination.

Key words: Barr Body, gender, forensic science.

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INTRODUCTION

Establishing the gender of a person is of paramountimportance 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 ofthe body or on harmful weapons at a crime prospect as well as at disaster sites can also be used for gender identification.[1]

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. The sex chromatin or Barr body is a condensation of chromatin present at the nucleus of cells in female individuals.[2] Their observation is possible in different cell types and is used for the rapid diagnosis of biological sex. 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.[3] 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]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.[5] 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.[6]The present study was conducted to assess accuracy of Barr bodies in sex determination.

MATERIALS & METHODS

The present study comprised of 80subjects of both genders. The consent was obtained from all enrolled patients.

Data such as name, age, gender etc. was recorded. 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. The cellular material was quickly smeared on the slide and was fixed immediately with 90% ethyl alcohol for 15- 30 minutes.Papanicolaou Staining was performed. 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).Data thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

RESULTS

Table I Distribution of patients

Total- 80		
Gender	**Males**	**Females**
Number	35	45

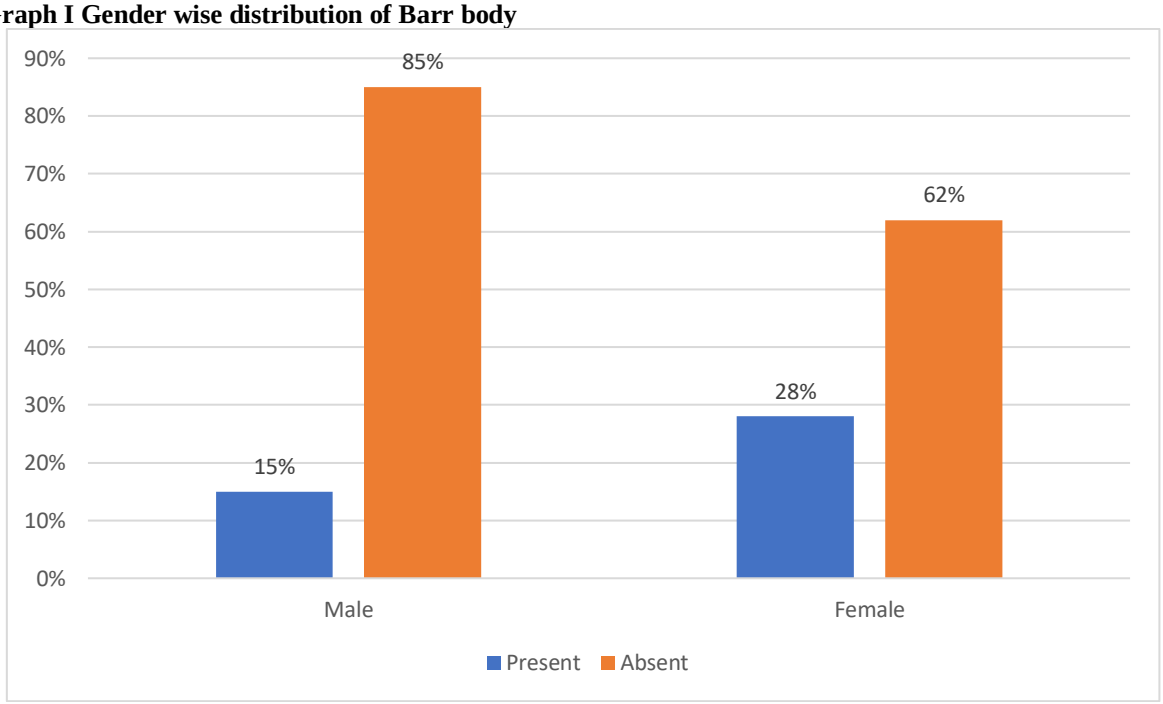
Table I shows that out of 80 subjects, males were 35 and females were 45.

Table II Gender wise distribution of Barr body

Gender	Present	Absent	P value
Male	15%	85%	0.01
Female	28%	62%	0.02
P value	0.05	0.04	

Table II, graph I shows that Barr bodies were present in 15% males and 28% females and absent in 85% males and 62% females. The difference was significant (P< 0.05).

Graph I Gender wise distribution of Barr body



DISCUSSION

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. However, PCR and karyotyping are very expensive and are not feasible for use.[7] Thus, Barr body demonstration for gender determinationusing exfoliative cytology is considered one of the simplest and easiest methods.[8] 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 absentin males.[9] The distribution of Barr body presentin 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.[10]The present study was conducted to assess accuracy of Barr bodies in sex determination.

We found that out of 80 subjects, males were 35 and females were 45. Archana et al[11]determined 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. 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. 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.

We observed that Barr bodies were present in 15% males and 28% females and absent in 85% males and 62% females. Kaur et al[12]evaluated 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 was concluded that buccal Barr bodies have the accuracy (54%). Hence it can be one of the reliable parameters in forensic odontology for sex determination.

Galdames et al[13]determined the effect of high temperatures on the diagnostic performance of the Barr chromatin observation on teeth. This study used 50 healthy teeth from 25 male and 25 female individuals aged between 14 and 44 years. The teeth were divided into 5 groups (each group with 5 female and 5 male teeth) and were exposed to controlled temperatures of 200, 400, 600, 800, and 1000 degrees C for 5 minutes. The coronal pulp was obtained and the tissue was processed and stained with hematoxylin-eosin. Four histological slides of male and 4 of female individuals were randomly selected, for each temperature level, which were observed by conventional microscopy at 100X magnification, each showing 50 cells per plate. The presence of 1 cell with visible sex chromatin was considered positive for females. It was only possible to evaluate the samples from groups subjected to 200 and 400 degrees C. In the groups analysed, the test showed 100% accuracy. The average number of cells found to be positive Barr chromatin was 15 at 200 degrees C and 11 at 400 degrees C. Hence, it was possible to detect the sex at these temperatures by observing chromatin of the Barr body in dental pulp.

In a study done by Datar et al[14], PAP stain was used in which the range of Barr-body-positive cells was observed as 4–20 in females and 0–5 in males. Reddy et al[15] examined mucosal samples stained with acetoorecin for the finding of Barr-body-positive cells and found out that female sample showed 18–72% cells showing Barr-bodypositive cells whereas male samples showed 1–3%.

Usually, demonstration of Barr body is superior when the nuclear staining is light, because in light nuclear background the deeply stained Barr body is seen prominently. Demonstration of nuclear membrane is noble when the nuclear membrane is preserved and smooth.[16]

CONCLUSION

Authors found that Barr Body estimation through buccal mucosal scraps can be a reliable method for sex determination.

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