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Determination of Age and Development of Barr body in Root Sheath Cells of Human Females

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Abstract

Hairs are potentially ubiquitous types of trace material which is found in most of the violent offences such as murder, assault and rape etc. Barr bodies can be seen in hair root sheath cells. The presence of Barr bodies indicates that sex of the person as female. The present study was intended to study the age of a person and percentage of Barr bodies in different age groups of human females.

Material and methods: 50 rooted hair samples were collected from the students of different age groups. The collected hair samples were stored in sterile plastic bags and were labeled. Hairs for analysis were taken out from the plastic bags using the forceps. Samples were cleaned with ethyl alcohol and hair bulb was cut with help of scalpel and the hair was put on slide and stained with aceto-orcein stain. Hair root sheath cells after staining were studied for Barr bodies under the microscope.

Results: In the present study, 50 hair samples were studied to know that how hair can be used in forensic investigations in determination of age through Barr bodies present in the hair. Mostly (33%) Barr bodies were observed in age group between 26-30 years and found absent below eight years of age and above 40 years of age.

Conclusion: Variations in the percentage of Barr bodies varies with age in human females and thus plays a very important for determination of age. This is important for medico legal purpose.

Keywords: Hair; Root sheath; Barr body; Aceto-Orcein Stain

Introduction

Current methods of forensic hair analysis rely upon comparisons of either hair morphology or DNA. Microscopic examination of hair can indicate species, race, body and method of removal. However, determining the age and gender of a subject based on hair morphology is not widely accepted. In forensic medicine sex from hair can be determined in decomposed bodies and mutilated bodies. Root sheath cells are resistant to autolysis; hence sex determination can be done even in decomposed bodies. The Barr body was first found by Barr and Bertram in the nuclei of the nerve cells of cats.

Hair may be present in all most all type of crime scenes and mostly in violent offences such as rape, assault, murder and road accident. Hair is trace evidence and it is crucial for solving criminal case to determine sex of a person in many situations. When hairs are present as circumstantial evidence these can help in solving case when it may not be possible from any other evidence. In humans, hairs found on the head, pubic region, arms, legs, and other body areas have characteristics that can determine their origin. Hairs can be transferred during physical contact, their presence can associate a suspect to a victim or a suspect/victim to a crime scene. The types of hair recovered and the condition and number of hairs found all impact on their value as evidence in a criminal investigation.

The sex chromatin is seen in inter phase nuclei of human beings and other mammals. It is one X chromosome heavily condensed along its entire length. Cajal was first to describe the presence of paranuclear mass in cats and human beings [1]. Later on it was described by Barr and Bertram who demonstrated the existence of sex chromatin in the cells of mucosa of cheeks of normal human females. This discovery offered an important diagnostic technology to the burgeoning clinical science community engaged with the medical interpretation and management of sexual anomalies. For determining the sex of a person, root sheath of the hair of a person can be used as this is

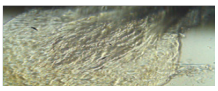
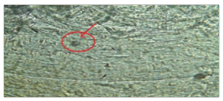
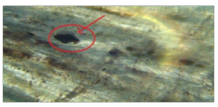
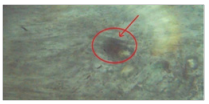
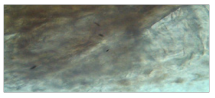
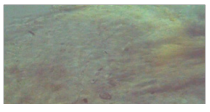
easy and non-invasive method. Barr bodies identify female sex and male sex is identified by the presence of florescent Y Bodies [2].

The Barr body in female inter phase nuclei is characteristically found as a darkly staining nuclear inclusion commonly associated with the nuclear membrane [3]. In cells carrying a diploid complement of auto some, X inactivation and Barr body formation occurs according to the N-1 rule: cells maintain a single active X chromosome and inactivate and condense all remaining X chromosomes [4,5]. The inactivated X chromosome is called a Barr body and is sometimes referred to as sex chromatin. Barr body can be obtained for study from buccal smear, pulp tissue, vaginal smears and hair follicle [6]. Gender determination of living person is required in doubtful cases like in sports, with altered physical and sexual features and to decide certain civil rights reserved for one sex [7]. Hair from a living female could be useful for determination of sex by studying Barr body at least up to three months after its collection and hair from a female dead body at least up to one week after its collection [8]. The Barr body remains within cells even though it is not genetically active. It can easily be viewed under a microscope when female cells are stained. The Barr body or sex chromatin displays itself as a richly stained circular disk.

Material and Methods

50 rooted hair samples of different age groups were collected from the students of schools and colleges at Allahabad, Uttar Pradesh, India after taking their consent. In the present study, we used plucked hair from the scalp of each female. The plucked hairs were stored in sterile plastic bags and the plastic bags were labeled. Hairs for analysis were taken out from the plastic bag using the forceps and samples were cleaned with ethyl alcohol. The hair bulb was cut with help of scalpel and the hair was put on slide. Staining solution was prepared from the stock aceto-orcein solution.55 a part of the distilled water was added to 45parts of the stock aceto-orcein solution. The staining solution was filtered at time of staining of the slides. Finally, the slide was examined with 40x or100x oil immersion objective lens on the immersion microscope for the presence of Barr bodies.

Table 1: Nature of female hair Barr bodies according to age.

Sr. No.	Age group	No. of hair sample	Status of Barr body	Status of Barr body
1	3-8 year	7		Absent
2	9-12 year	4		Present
3	13-19 year	6		Present
4	20-25 year	8		Present
5	26-30 year	7		Present
6	315-35 year	5		Present
7	36-40 year	4		Present
8	41-44 year	4		Absent
9	45-50 year	5		Absent

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Results

50 rooted hair samples of females of different age groups were collected from the students of schools and colleges. In this study, hair samples were stained with aceto-orece in for detection of Barr body in different age group of females. In our study, the presence of Barr bodies was different in different age group of females. In 3-8 years age group of females, 7 samples were taken for study and all were negative for the presence of Barr body. Barr bodies were found to be present in 34 samples in the age group of 9-40 years of females. Barr bodies were not found above 40 years of age group in 9 samples (Table 1).

Table 2: Percentage of Barr bodies in different age groups.

Age Groups	No. of hair samples	Percentages
3-8 year	7	0%
9-years 12	4	6%
13-19 year	6	20%
20-25 year	8	22%
26-30 year	7	33%
31-35 year	5	15%
36-40 year	4	4%
41-44 year	4	0%
45-50 year	5	0%
Total	50	100%

In the present study, the percentage of Barr bodies was found different in different age group of females. In 3 to 8 years age group of females, 7 samples were taken and percentage was 0. In 9-12 years age group of females, 4 samples were taken which showed 6% of Barr bodies. In 13-19 years age group of females, 6 samples were taken which showed 20% of Barr bodies. In 20-25 year age group of females, 8 samples were taken which showed 22% of Barr bodies. In 26-30years age group of females, 7 samples were taken which showed 33% of Barr bodies. In 31-35 year age group females, 5 samples were taken which showed 15% of Barr bodies. In 36-40 year age group of females, 4 samples were taken which showed 4% of Barr bodies. In 41-50 years of age group, 9 samples were taken in which the Barr bodies were found negative and thus percentage was 0 (Table 2).

Discussion

In our study percentage of Barr bodies' variations was noticed in range of 6-33 % of females which is consistent with the study conducted by Singh et al. [9] and Chakrabarti and Momonchand [8]. Our study is not in accordance with the study made by Nagamori et al. [10] who detected Barr bodies in both buccal smears and hair cortex using AF Schiff stain and found that in female samples the frequencies were 58–87% (71.3% in average) in the cells of buccal mucosa and 59-85% (67.1% in average) in the nuclei of the hair cortex.

Our study is in accordance with the study of Baby et al. [11] who reported that in buccal smears of 30 females in the age group of 16-60 years, the Barr bodies were seen positive in AF Schiff stained cells ranged from 16% to 53% and PAP stained positive cells ranged from 9% to 38% and Shankar et al. [12] who reported sex chromatin variation within range from 20-52% in the age group of 12 years to above 60 years. In our study, the percentage of Barr bodies variations in hair root sheath cells were seen increasing with age up to 30 years as 0 % in 3-8 years age group, 6% in 9-12 year age group, 20 % in 13-19 years age group, 22 % in 20-25 year age group and 33 % in 26-30years age group which is consistent with the study done by Singh et al. [9].

With increase in age above 30 years the percentage of Barr bodies starts to decline as 15 % in 31-35 year age group, 4 % in 36-40 year age group and 0% above the 40 years of age group which is consistent with the study conducted by Singh et al. [9] wherein the percentage of Barr bodies variations with age group almost occurred in same pattern.

Conclusion and Summary

Since Barr bodies are present within nuclear material, special stains for nucleus such as papanicolaou stain, feulgen and guard stains, orcein, hematoxylin and eosin, cresyl violet, carbolfuschin, and fluorescent staining are used to visualize them. Barr bodies are important for age and sex determination. Variations in the percentage of Barr bodies varies with age and thus plays a very important role for age calculation and important for medico-legal purpose. Under various pathological conditions, there are alternation in number and size of Barr-bodies, which produces negative results and produces difficulty in sex determination. Advanced techniques such as in situ hybridization, immunofluorescence method can be used for determination of the sex of the individual.

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