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Original Research Paper

Anatomy

A STUDY OF BARR BODIES IN CASES OF PRIMARY AMENORRHEA.

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ABSTRACT

BACKGROUND: Barr body (or) X- chromatin is a heterochromatin mass seen in all somatic cells of females species. They account to nearly 80-90% in females and 1-3% of cells of normal males. Primary Amenorrhea a clinical condition is of varied aetiology, however Genetic factors being the major cause. Either a structural or a numerical anomaly like X- monosomy of a female results in failure of commencement of menstruation. Identification of chromatin negative condition in patients of primary amenorrhoea constitutes my study. **METHODOLOGY:** The study is conducted on 58 patients who visited Obstetrics Gynaecology clinics Visakhapatnam district with presenting complaint of primary amenorrhea. Buccal smear examination is done to all the patients and observed under the microscope for Barr bodies. Photographs were taken and the observations were tabulated and analysed. **RESULTS:** Absence of Barr Body was observed in 28 cases (chromatin negative) and 26 cases were chromatin positive and 4 cases showed mosaicism. **CONCLUSION:** Primary Amenorrhea due to chromosomal aberrations is a serious condition as it is associated with intense psychological trauma along with physical. In Turner's syndrome single X-chromosome is present (45XO), the subject is female in phenotype, but the ovaries are rudimentary (Streak Gonads) and absence of development of secondary sexual characters. So buccal smear is a simple, rapid test that will enable us to decide which patients are to be referred for further investigations to confirm the diagnosis.

KEYWORDS : Barr Bodies, X- Inactivation, Buccal smear, Chromosomal Aberrations, Primary Amenorrhea

INTRODUCTION:

The relative tolerance of the human karyotype for X-Chromosome abnormalities can be observed and explained in terms of X- Chromosome inactivation.[1]

X- Inactivation is that seen in somatic cells of normal females, one of the X-Chromosome is inactivated early in development, thus equalising the expression of X- linked genes in both sexes.[2] In normal female cells, the choice of which X chromosome is to be inactivated is random. It can be either maternal X or paternal X. Once the Inactivation takes place it is then maintained same hence forth, so females are considered as mosaic with respect to X-linked gene expression.[3]

Inactivated X Chromosome otherwise called as Barr bodies are seen as Plano convex disc like heterochromatic mass seen adherent to inner nuclear membrane of nuclei of all somatic cells.

SEX CHROMATIN STUDIES:

The inactivated X chromosome known as the Barr body or sex chromatin can be identified in a significant proportion as a condensed planoconvex disc of genetic material adherent to the inner wall of nuclear membrane of the nuclei of the cells in all tissues of the human female. The inactivation of X chromosome is due to methylation of cytosine to 5 methyl cytosine at certain sites in the DNA, accounting for altered gene activity leading to X chromosome inactivity.[4]

Lyon's Hypothesis (Mary. F. Lyon 1962):

1. According to her hypothesis inactivation occurs early in embryonic life.[5]
2. Inactivation is random but fixed. The inactive X can be maternal or paternal (Xm or Xp) in different cells of the same individual.
3. Sex chromatin is detected in blastocyst at 9—12 days and in embryo proper it is demonstrated after the 18th day. (This feature enabled the modern Gene cists to research on X Chromosome Reactivation)

Genetic Significance of X Inactivation:

1. Dosage compensation
2. Variability of expression
3. Mosaicism

The total number of Barr bodies is always one less than the total number of X chromosomes present in the karyotype. If the chromosomal constitution is 45 X, then the patient though phenotypically female is Sex Chromatin Negative. Investigation of interphase nuclei allows provisional designation of the sex chromosome status. Since this can be done with easily accessible method, buccal cells are studied to identify the Barr bodies it is indicated whenever an anomaly of the sex chromosomes is suspected. The size of the sex chromatin body varies from the normal 46 XX karyotype sex chromatin body in cases where structural anomalies of the X chromosome exist in the karyotype.[6] Where there is a ring chromosome formation of the X chromosome, the sex chromatin body is slightly smaller in size. The deletions of the long and short arms of the X chromosome result in Sex chromatin body being smaller than normal. Bigger sex chromatin bodies are seen when isochromosome formation of the long arm of the X chromosome takes place. [Barr body; source, BIODIDAC, University of Ottawa Dt: 7.12.07]

AIM & OBJECTIVE:- Study of Barr bodies in females presenting with primary amenorrhea

This study was conducted on 16 to 20 year old females who have not attained menarche, referred form all Obstetrics and Gynaecology departments and OPDs along the northern coastal region of Andhra Pradesh.

MATERIALS AND METHODS:

Buccal smear examination

Lab material required

1. Glass slides.
2. Wooden or plastic spatula
3. Methylene blue and H & E stain
4. Distilled water
5. Isopropyl alcohol

Collection of sample: The following steps have been taken to ensure to get proper smear which will enable us to interpret the results accurately.

1. Oral cavity examined for any aberrations or ulcers.
2. Oral gargling is encouraged to be done thoroughly to clear the mouth of food debris.
3. Buccal mucosa scraped gently by spatula.
4. Smeared evenly over the glass slide.
5. Air dry the slide.
6. Fixation with isopropyl alcohol is done.
7. Stains used are H& E stain; Methylene blue.
8. DPS Mount done and cover slip is placed.
9. Observation under 10X, 40X & 100X

The sex chromatin studies are done from buccal smears. Epithelial cells are obtained by scraping the buccal mucosa with a glass slide and this material is spread over a small area of the clean slide and fixed immediately in isopropyl alcohol to water and then rinsed. It is then stained in methylene blue and observed under a microscope. The sex chromatin count is per 100 buccal mucosal cells.

RESULTS: -

In the present study on careful examination
Out of the 58 cases of primary amenorrhea
No.of cases with chromatin positive are 26 accounting to 44.82% (Table 1)

Table 1: Sex chromatin studies in 58 primary amenorrhea cases

S.No	Sex chromatin	No. Of cases	Percentage
1	Positive	28	48.27
2	Negative	27	44.82
3	Mosaics	04	06.89

No.of cases with chromatin negative are 28 accounting to 48.27%

No.of cases with mosaicism i.e. 4 accounting to 06.89%

Table 2: Sex chromatin studies in 04 cases of mosaic cases out of 58 primary amenorrhea cases

S.No	Case No.	Percentage of sex chromosome positive	Percentage of sex chromosome negative
1	14	81	19
2	39	73	27
3	41	60	40
4	47	77	23

The Sex Chromatin studies in 04 Mosaics out of 58 Primary Amenorrhea cases have been presented in the Table 2. The overall mean for the Sex Chromatin negative cells is 27.25, this is expected among the mosaic cases and the overall mean for the sex chromatin positive cells is 72.75

DISCUSSION: -

Cytological tests and chromosomal sex were available from 1956. Barr and Bertram (1949) demonstrated the presence of a small chromatin body in contact with the nuclear membrane visible in 20-90% of somatic cell nuclei of females [2] and seen only in 1-3% of cells of normal males. When Barr's nuclear chromatin was available, it was found that most patients with Turner's syndrome were chromatin negative and they were considered as genetic males.

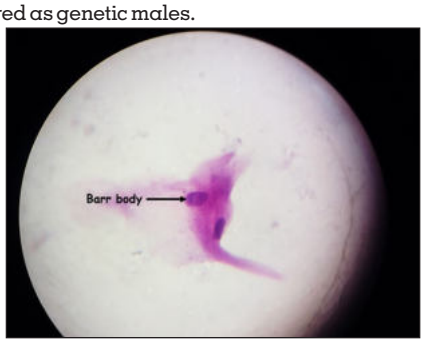

Figure 1: Buccal cell with the nucleus showing Barr body

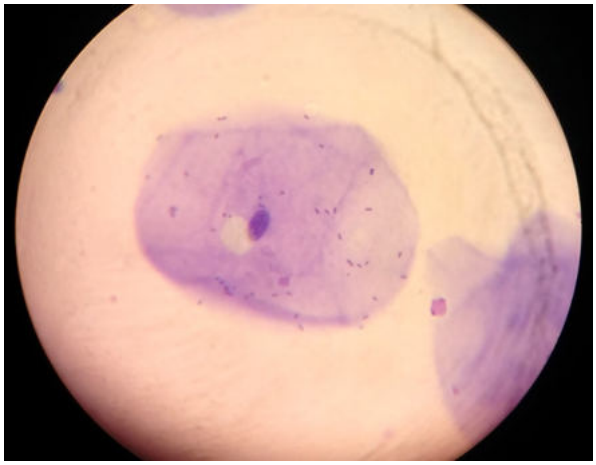

Figure 2: Buccal cell with the nucleus showing no Barr body

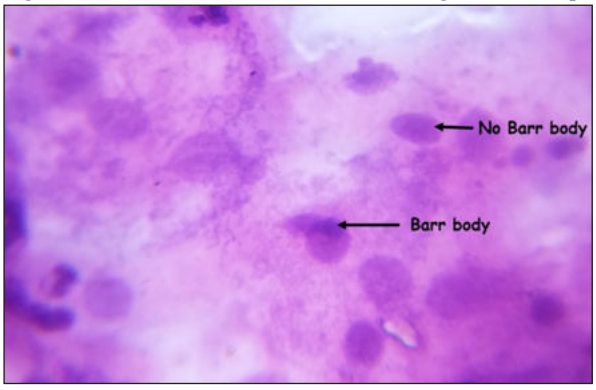

Figure 3: Mosaicism case with few cells showing Barr body and few with absent Barr bodies

The study of sex chromatin in humans reveals the status of X chromosome (both numerical and structural) therefore this technique is conveniently employed in children with external ambiguous genitilia, girls with primary amenorrhea, sterile women and men. Even though the study is preliminary and it needs confirmation by further chromosomal studies. A humble attempt has been made in the present study to see how many are sex chromatin positive, negative and mosaics. The results are tabulated in (table 1). It is analysed that out of the 58 cases 26 cases are chromatin positive (44.83%)(Figure 1) and 28 cases are chromatin negative (48.27%)(Figure 2), mosaics are 4 (6.89%)(Figure 3) and positive Barr bodies in 1 case. The study on Primary Amenorrhea in year 1996 Lakshmi et al done on 70 Primary Amenorrhea cases revealed 64 cases (91.42%) chromatin positive and 6 cases are negative (8.57%) and mosaics in 11 cases (15.71%). The overall mean for sex chromatin positive cells is 69.77. No much extensive studies have been done in these areas.

CONCLUSION: -

Genetic factors are being the major cause of primary amenorrhea. This study helps us to screen preliminarily the cases of primary amenorrhoea related to genetic diseases like Turner's Syndrome. However the chromosomal aberrations could be either numerical or structural can be analysed only by further examinations like karyotyping. Menarche and the regular menstrual cycle signal an unintentional completion of the pubertal growth process. Menarche usually happens between the age group of 10 to 18 years, timely inception of menstruation provides an important landmark in pubertal development. It ensures neuro endocrine, gonadal and anatomical components of the reproductive system are intact and mature. Failure of attaining menarche beyond sixteen years is referred to as primary amenorrhea. In general it is often associated with disorders of endocrine function with gonadal and somatic anomalies, as well as chromosomal

aberrations, so buccal smear examination enables us to identify chromosomal abnormality.

Barr body study by the way of buccal smear examination is the fastest, cost effective, simple technique of preliminary examination of chromosomal disorders and genetic sex determination. It is highly useful in Primary Health Centers, Community Health Centers, during Medical camps and Area Hospitals as outpatient investigation. Individuals and family members can be counselled affirmatively regarding further investigations that have to be done henceforth, we can encourage these patients to go for further confirmatory test like karyotyping. The rest of the patient who are chromatin positive can be counselled accordingly.

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