

Buccal Smear Examination for Barr Bodies as Sex Determination Tool: Is it a Redundant Exercise?

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Abstract

Neuroanatomists Murray Barr and Ewart Bertram in 1949 provided first study on sex chromatin, later named as Barr body. This led to a theory proposed by Mary F. Lyon in 1961 called as "Lyon hypothesis". Since then the Barr body from buccal smear is used as a sex determination tool. However, with advent of modern molecular methodologies it is labeled as a useless tool by some investigators. Authors of this article wish to stress the importance of Barr body examination as a low cost effective tool.

Keywords: Sex Chromatin; Barr Body; Amenorrhoea; Inguinal Hernia; Turner Syndrome.

Introduction

Neuroanatomists Murray Barr and Ewart Bertram in 1949 provided first study for a difference between cells of male and female individuals of the same species. They discovered a nuclear body that was present only in female and not in male neuronal cells in cats, which they termed "sex chromatin" and which is famously called "Barr body" after Murray Barr [1]. It is the inactive X chromosome seen in interphase nucleus of female somatic cell [2].

Mary F. Lyon in 1961 put forward a hypothesis. She proposed the concept of random X chromosome inactivation as the mechanism of dosage compensation in female mammals. Her hypothesis is known as "Lyon hypothesis" [3].

Inactivation of X Chromosome in Females [4]

The X chromosome to be inactivated is randomly chosen in somatic cells. But once decided whether the paternal or maternal X chromosome will be inactivated all the cells follow the inactivation process. Spontaneous, unprogrammed reactivation is extremely rare. The gene for inactivation process is

located on long arm of X chromosome. The centre is called X inactivation centre.

The inactive X chromosome is distinguished from the active X chromosome by the following characteristics: (i) Overall transcriptional inactivation (apart from certain X-linked genes which escape inactivation and the XIST gene – X Inactive Specific Transcript); (ii) Heterochromatic condensation at interphase of the cell cycle, sometimes visible as the Barr body; (iii) Late replication during S phase; (iv) DNA methylation of cytosine residues at CpG dinucleotides in the region of X-linked genes; (v) Hypoacetylation of histone (vi) Expression of the XIST gene located at the X-inactivation center.

The Mechanism of X Chromosome Inactivation [5]

The basic mechanism appears to be DNA methylation. Literature has reported that CpG islands of several genes are methylated on the Xi (inactive X) chromosome and unmethylated on the Xa (active X chromosome).

Turner Syndrome and Klinefelter's Syndrome [6]

Turner syndrome patients inspite of having female phenotype have Buccal smear negative for Barr body. On the other hand Klinefelter's syndrome (47, XXY) patients are phenotypic males with one extra X chromosome and hence Barr body positive.

Inguinal Hernia in Children and Barr Body [7, 8]

Few authors have reported that female children

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with inguinal hernia should raise a suspicion about child's nuclear sex as about 2% female children with inguinal hernia have been reported to have intersex differentiation syndrome.

Ambiguous Genitalia and Barr Body [9]

Studies suggest that female children presenting with Ambiguous genitalia as a result of genital virilization may have congenital adrenal hyperplasia (CAH). This is due to 21-hydroxylase deficiency. Authors have recommended having prenatal genetic diagnosis of CAH and fetal sex determination to identify affected female fetuses at risk for genital virilization so that prenatal dexamethasone treatment can be administered early in the first trimester.

The Positive, and Negative Barr Body [10]

A buccal smear showing 20-50% cells having Barr body is said to be positive smear. A normal female will show buccal smear positive for Barr bodies. A buccal smear from a normal male will show 2-5% cells with Barr bodies. This smear is said to be Negative for Barr bodies.

Barr Body Size and Numbers [11]

As evident from literature Buccal smears showing Barr bodies in the range between 5-20% along with small size of Barr bodies should raise suspicion of chromosomal abnormalities like deletion of a part of X chromosome.

Extra number of Barr bodies in females and males is suggestive of presence of extra X chromosome, like in case of Females having 47,XXX complement may present with menstrual disturbance and absence of secondary sex characters.

The frequency of Barr bodies is decreased during pregnancy as well as in women on oral contraceptive pills as reported in a few studies [12].

The Y Chromosome and F Body [13]

In a male, a smear of buccal mucosal scraping or peripheral blood stained with dye quinacrine hydrochloride will show bright fluorescent dot in the nucleus indicating presence of the Y chromosome which selectively takes up this dye, which has the property of fluorescing strongly under ultraviolet light.

Twenty years after the paper of Barr and Bertram, the fluorescent (F) body, or Y chromatin was discovered in interphase nuclei [14].

Limitations of Barr Body Studies [15]

Literature mentions that the buccal smear, as a test for sex chromatin, is of no use to the clinician investigating a reproductive problem. Irrespective of results of Barr body authors also suggest that it is essential to do full chromosomal analysis and the sex-chromatin test is of use only to the cytogeneticist to complement his or her laboratory studies. The presence and frequency of Barr bodies is helpful in deciding whether the possibility of sex-chromatin mosaicism is great enough to warrant cytogenetic analysis of more cells or examination of other tissues.

Materials and Methods

This study includes patients referred to this department between January 2008 to December 2012 for Barr body in buccal smear for determination of sex. This investigation is done routinely in this department and is done free of cost.

The patients having Amenorrhoea, ambiguous genital and inguinal swellings were referred from Gynecology, Pediatrics and Pediatric surgery departments.

The referred patients were re-examined in Anatomy department. All the findings and results of investigations were entered in the register maintained in the department for buccal smear studies.

The patients were instructed about the procedure.

After the patient had rinsed mouth thoroughly with water, Inner aspect of cheek was scraped gently with a blunt spatula. The superficial cells were taken on a sterile slide. The smear was spread evenly on slide and kept for drying. Then slide was processed as given below:

It was placed in 70% Alcohol solution for 5 minutes.

Rinsed in Distilled water.

Placed in 1N HCL for 5 minutes.

Again rinsed in Distilled water.

Stained in 5% Toluidine blue for 3-10 minutes.

Washed in slow running water.

Air dried.

Observed under high power and oil immersion lens.

The Barr body appears as mass of chromatin attached to inner aspect of nuclear membrane in the cell. Only those bodies which were situated at the periphery of the nucleus were considered to be Barr

bodies because human mucosal nuclei has high occurrence of other non-sex bodies present freely in the nucleoplasm, so can be confused with Barr body, also maximum studies show the occurrence of Barr body at periphery in human nuclei [16].

All patients were advised Karyotyping and FISH to confirm the results and decide line of treatment. In spite of best efforts it was difficult to follow up the patients later.

Results

From observation table it is seen that buccal smear examination for Barr Body was advised as a primary tool of sex determination as it is noninvasive and less

costly compared to Karyotyping or other molecular genetic studies. Also those cases having clinical features suggestive of some genetic abnormalities were referred for evaluation.

The distribution of types of cases referred to the department was as follows:

Swelling in groin or inguinal hernia (case numbers 4, 5, 6, 19, 20, 21, 22, 24, 25 = 9 cases).

Amenorrhea (Primary or secondary) was the second most common presenting complaint seen in 8 cases (case numbers 1, 2, 3, 7, 8, 10, 12, 14).

Ambiguous genitalia (3 cases), Congenital heart disease, Lymphadenopathy, Hypospadias, Turner Syndrome, Poor scholastic performance and delay in development were other presenting complaints.

Observation Table

Sr. No.	Age	Sex	Presenting complaint	Investigations	Buccal Smear Examination
1.	18	F	Primary. Amenorrhea	Ut- normal Ovaries not commented on	Positive
2.	20	F	Primary. Amenorrhea	Ovaries N Ut- Bicornuate Blind vagina	Positive
3.	17	F	Primary. Amenorrhea	No sec. Sex Characters. Ut- not palpable,	Negative
4.	1 1/2	F	Left Inguinal hernia	Ext. Genitals infantile, Short stature Nose bridge flat No Turner stigmata	Positive
5.	3 1/2	F	Right Inguinal hernia	Short neck, Low posterior hairline, widely spaced 2 nd toe Arm span > height	Positive
6.	17	F	Right Inguinal hernia	1 st cousin marriage in parents Hypoplastic uterus	Positive
7.	18	F	Primary Amenorrhea	Ut and ovaries not visualized Sec. sex characters well developed	Negative
8.	18	F	Primary Amenorrhea	Hypoplastic uterus, Ovaries - normal	Positive
9.	4	F	Ambiguous genitals	Bilateral empty scrotal bags, Low set ears, Flat nose bridge	Positive
10.	18	F	Secondary Amenorrhea	Flat nose bridge, Short neck, Low posterior hair line, Arm span < height, Consanguinity	Positive
11.	8	F	Lymphadenopathy	Broad short neck, Increased blood flow in kidneys, Prominent hepatic veins, ascites, Coarctation of aorta, Splenomegaly.	Positive
12.	18	F	Primary Amenorrhea	Ut and Ovaries seen, Breast developed, no axillary hairs, Pubic hairs sparse, Ext. Genitals infantile	Positive
13.	12	F	CHD with short stature	Coarctation of aorta, Low set ears, Low posterior hairline, Flat nasal bridge, Axillary hairs absent	Positive
14.	16	F	Prim Amenorrhea	Rudimentary uterus, No vagina, blind pouch, Rectum and bladder adherent, Low set ears, Low posterior hairline, Cubitus valgus	Positive
15.	13	F	Poor scholastic performance	Short stature, Palmar creases are scanty, widely spaced nipples, High arched palate, Consanguineous	Positive
16.	9	F	Hypospadias with chordee	No uterus, ovaries, Undescended testis, well defined hypochoic tissue in rectovesical pouch.	Negative
17.	12	F	Developmental delay	Ut and ovaries seen, Lt hydronephrosis with hydroureter, short stature, Neck webbed, No axillary or pubic hairs	Positive

18.	4	F	Ambiguous genitals	Ut seen, Ovaries normal, Enlarged clitoris, congenital adrenal hyperplasia	Positive
19.	8	F	B/L Inguinal hernia	Rt swelling smaller than left, Left has cough impulse	Positive
20.	5 1/2	F	B/L Inguinal hernia	Bilateral inguinal swellings, cough impulse +, Ext genitals normal	Positive
21.	3 1/2	F	Lt. Inguinal hernia	Swelling in left groin region which increases with straining and coughing	Positive
22.	11 month	F	Lt. Inguinal swelling	Cough impulse positive	Positive
23.	11	F	Turner Syndrome	Short stature, Neck webbing, Increased carrying angle, widely spaced nipples	Positive
24.	7	F	B/L Inguinal hernia	Cubitus Valgus	Positive
25.	6	F	B/L Inguinal hernia	Bilateral groin swelling	Positive

Abbreviations: F= Female, Ut= Uterus, Rt=right, Lt = left, CHD = Congenital heart disease, B/L= bilateral

Discussion

It is seen from the observation table that the cases presenting with primary or secondary amenorrhoea have characteristic clinical findings. Of the 8 patients of amenorrhoea, case no. 3 and case no. 7 have buccal smear negative for Barr bodies. Looking at their clinical features case no 3 (who has Primary amenorrhoea, short stature, absent secondary sexual characters and infantile external genitals) and case no 7 (Uterus and Ovaries not visualized on Sonography) which is suggestive of 46, XY gonadal dysgenesis [17]. In such cases, it will be a futile exercise to send these patients for expensive investigations like Karyotyping and FISH when buccal smear examination can give valuable insights in planning treatment for such patients.

Case No.11 (Broad, short neck, coarctation of Aorta), 13 (CHD with short stature), 17 (short stature, webbed neck, absent pubic and axillary hairs), and case no. 23 (short stature, webbed neck, increased carrying angle, widely spaced nipples) have features suggestive of Turner phenotype [18] and the positive Barr body can be due to presence of mosaicism [19].

Case No. 15 is a phenotypic female with short stature and scanty palmar creases. As discussed earlier the short stature is indicative of Turner phenotype.

Case No. 16 with hypospadias and chordee is a case of wrong gender assignment as it is clearly evident by presence of undescended testis, absence of uterus and ovaries and a buccal smear negative for Barr body.

So as per authors of this study, this primary noninvasive and cost effective tool eliminates the need for detailed chromosomal analyses for sex determination in many cases e.g. in sports or intersex disorders where clinical features and Barr body examination correlation can establish a diagnosis. However due to availability of modern molecular diagnostic techniques this tool has been used less

frequently and as specified earlier by some authors [20] it has been awarded only academic value.

Since Mary Lyon's hypothesis, a lot of progress has been made towards the understanding of this fundamental process of Barr body formation. Yet many things are left to be discovered. For instance correlation of Barr bodies and cancer [21].

It is also interesting to note that there are conditions under which this inactivated X chromosome in females becomes activated again. Physiological stress is one such condition reported by some investigators [22]. The part of X which carries the gene XIST required for inactivating X chromosome assumes essential role. It is left to be investigated if deletion of this gene will lead to failure of X inactivation in females and leading to genetic disorder.

From this study the corresponding author wants to suggest that even if modern techniques are made available for diagnosing clinical cases where sex determination is required, the Barr body examination can serve as a low cost and non invasive tool to supplement other investigations like Karyotyping. A further research is required to know if the Barr body shape and size changes with hormonal influence in menstrual cycle and if this tool can be used as a screening measure for early detection of cancers.

Conclusion

The role of this investigation as a diagnostic tool cannot be undermined so far as sex determination is concerned.

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