Barr Bodies and Sex Identification in Intersexed: Current Concepts

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ABSTRACT

The discovery of Barr body offered an important diagnostic technology for medical interpretation of sexual anomalies, such as in intersexuals and transsexuals. Establishing individuality and identification of sex becomes important in several situations like in gender assignment for receiving specific civil rights for transgenders, legitimacy to partake in competitive sports or even in forensic situations. In spite of so much importance being attached to sex identification, very little work has been attached to sex identification, very little work has been done in India about study of sex chromatin in buccal smears in the intersexed. This review emphasis the need to primarily understand the biology of intersexed individuals, current information of transgender rights in India and the role of cytological evaluation of Barr bodies in preliminary screening of individuals without invasion of privacy.

Introduction

Establishing individuality is imperative in any investigating procedure. Often, determination of the sex of an individual becomes important in following situations: for the purpose of simple identification in the living where the individual of one sex carries the features of the opposite sex; when a person appears to possess the primary sex organs of both the sexes; for the purpose of deciding whether an individual can exercise certain civil rights reserved for one sex only; for deciding questions relating to competing in sex specific athletic and sport events, legitimacy, divorce, paternity disputes, and also to some criminal offences and identification of sex of dead individuals in an advanced state of decay where primary sex organs are lost due to decomposition.[1]

In massive accidents and also in natural disasters, it becomes difficult to identify the bodies. In such instances, buccal smears could help in detecting the sex and thereby establishing the identity.[2]

Demonstration of nuclear sex plays a vital role as far as sexing of the individual is concerned. Nuclear sex can be demonstrated by the study of [1]:

Karyotyping: Direct study of type of sex chromosome in the cell-by-cell culture. This is expensive and is not feasible in all situations.

Fluorescent body (Y chromatin): A demonstration of nuclear fluorescent bodies that is Y chromatin indicates male. This definitely requires special stain and fluorescence microscope.

Polymerase chain reaction: Polymerase chain reaction to amplify DNA sequences of SRY gene, on the sex chromosome. This is similar to karyotyping, as it is not feasible in all situations. It is expensive and inferior to karyotyping but not superior to the chromatin test.

Barr bodies (X-chromatin): In contrast, the study of Barr bodies is advantageous in that it can be studied even under an ordinary compound microscope with simple staining techniques. The easily available material for Barr body studies is the buccal mucosa, which can be obtained without inflicting trauma on the subject. The buccal smear technique to identify sex was developed by Moore and Barr in 1955 [3].

Role of Cytodiagnosis of Barr Bodies

Barr bodies are known to arise from inactivation of X chromosome in a female cell [4]. Barr bodies are feulgen positive, heteropyknotic, and basophilic, intranuclear structures, seen in mammalian cells during interphase.

Most often, they are noticed as densely stained condensed chromatin masses adjacent to the nuclear membrane. They can be Plano-convex, biconvex, triangular, spherical, or rectangular in shape when observed under ordinary microscope in oil immersion. They measure about 0.8 to 1.1 µm in diameter.[1]

The Barr body is not present in the nuclei of males although they also have one X and Y chromosome because X in the males remains uncoiled (extended) in interphase nuclei [5] This discovery of Barr bodies also seemed to offer a new way to identify the true, underlying sex in those whose bodies or lives were sexually anomalous or intersexed individuals (currently classified under a umbrella term as 'transgenders').[6]

Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp.[7]

Sex identification in the medical science using sex chromatin has provided 'good enough' evidence, using simple evaluation of Barr bodies. Patients are encountered occasionally in whom the sex chromatin pattern is unlike that of normal individuals because of a variety of unusual sex chromosome complexes.[6]

The buccal smear test is preferred, because of its simplicity, for routine use as a diagnostic aid or when conducting mass surveys for research purposes.

Defining Some Common Terms

GENDER V. SEX

In everyday language as well as in the law, the terms "gender" and "sex" are used interchangeably. However, it is often important to distinguish the two terms. The term "sex" refers to a person's biological, anatomical, biochemical identity as male or female. The chromosomal sex refers to presence of XX (female) or XY (male) chromosome in the cells. The term "gender" is reserved for the collection of characteristics that are culturally associated with maleness or femaleness [8].

Gender Identity and Gender Expression

"Gender identity" refers to a person's internal, deeply felt sense of being either male or female, or something other or in between and not visible to others. In contrast, a person's "gender expression" is external and socially perceived. Gender expression refers to all of the external characteristics and behaviors that are socially defined as either masculine or feminine, such as dress, mannerisms, speech patterns and social interactions [9].

Intersex

An intersex person is born with sexual anatomy, reproductive organs, and/or chromosome patterns that do not fit the typical definition of male or female. This may be apparent at birth or become so later in life. An intersex person may identify as male or female or as neither. Intersex status is not about sexual orientation or gender identity: intersex people experience the same range of sexual orientations and gender identities as non-intersex people [10]

Biological sex in humans may be determined by five factors present at birth [9]:

- The number and type of sex chromosomes;
- The type of gonads—ovaries or testicles;
- The sex hormones;
- The internal reproductive anatomy (such as the uterus in females), and
- The external genitalia.

People whose five characteristics are not either all typically male or all typically female at birth are Intersex [11].

Transgender

Transgender has become an "umbrella" term that is used to describe a wide range of identities and experiences, including but not limited to: pre-operative, post-operative, and non-operative transsexual people; male and female cross-dressers (sometimes referred to as "transvestites,"); intersexed individuals; and men and women, regardless of sexual orientation, whose appearance or characteristics are perceived to be gender atypical. In its broadest sense, transgender encompasses anyone whose identity or behavior falls outside of stereotypical gender norms.[12]

Epidemiology

At least one in every 2,000 children is born with a sexual anatomy that mixes male and female characteristics in ways that make it difficult, even for an expert, to label them male or female. Although no one is ever born with two full sets of genitals, male and female, some intersexed infants may have ambiguous genitalia, such as a penis that is judged "too small" or a clitoris that is judged "too large" [10].

Intersex is a group of conditions where there is a discrepancy between the external genitals and the internal genitals (the testes and ovaries). But a lot more people than that are born with subtler forms of sex anatomy variations, some of which won't show up until later in life [11]
Intersex can be divided into four categories [10]:

- 46, XX Intersex
- 46, XY Intersex
- True Gonadal Intersex
- Complex or Undetermined Intersex

46, XX Intersex: These are genetic females (46XX) born with hypertrophied clitoris (to appear like a penis) leading to ambiguity of genitalia. The person has the chromosomes of a woman, the ovaries of a woman, normal uterus and Fallopian tubes, but external (outside) genitals that appear otherwise. This usually is the result of a female fetus having been exposed to excess male hormones before birth.

46, XY Intersex: The person has the chromosomes of a male and internally, testes may be normal, malformed, or absent. The external genitals are incompletely formed, ambiguous, or clearly female. This condition is also called 46, XY with under virilization [10]

5-alpha-reductase deficiency: People with 5-alpha-reductase deficiency lack the enzyme needed to convert testosterone to dihydrotestosterone (DHT). Some of the babies have normal male genitalia, some have normal female genitalia, and many have something in between. Most change to external male genitalia around the time of puberty [13]

Androgen insensitivity syndrome (AIS): This is the most common cause of 46, XY intersex (previously called as called testicular feminization). Here the hormones are all normal, but the receptors are insensitive.

Complete androgen insensitivity: As the external genitalia are completely feminine such a baby is not brought to the doctor at birth. Later on, presentation may be for inguinal hernia, or for primary amenorrhea at puberty. These children are best reared as girls as they cannot be distinguished from normal girls at all. The only surgery they require is gonadectomy for removal of testicular tissue and vaginoplasty.

True Gonadal Intersex: These children generally present with ambiguous genitalia and arriving at a diagnosis usually takes time, as gonadal biopsies with or without laparotomy/laparoscopy and karyotyping is required before conclusively proving the diagnosis. The person may have XX chromosomes, XY chromosomes, or both. The external genitals may be ambiguous or may appear to be female or male [10]

Complex or Undetermined Intersex Disorders of Sexual Development: Many chromosome configurations other than simple 46, XX or 46, XY can result in disorders of sex development. These include 45, XO (only one X chromosome), and 47, XXY, 47, XYY -- both cases have an extra sex chromosome, either an X or a Y. These disorders do not result in a condition where there is discrepancy between internal and external genitalia. However, there may be problems with sex hormone levels, overall sexual development, and altered numbers of sex chromosomes [8].

Children with intersex disorders and/ or with abnormal sex chromosomes are at an increased risk for development of malignancy, particularly in their gonads. It is seen more commonly in: complete AIS, with malignant germ cell tumours, usually in adult life, all gonadal dysgenetic disorders in patients having a Y chromosome, fertility is unlikely in many intersex conditions, with the exception of CAH.

Transgender and their Civil Rights

Transgender people experience a mismatch between their gender identity or gender expression and their assigned sex. The term originally referred to biological men who are satisfied with their male genitalia, but who wish to be seen and to live in the world as women. Such individuals prefer to undergo sex reassignment surgery and hormonal therapy to become a 'transsexual'. There are several other terms in India used to describe transgenders as **'Hijras', 'Aravanis and *'Thirunangi', 'Jogtas/Jogappas', 'Shiv-Shakthis'*** [14]

In India, transgenders face sexual discrimination, sexual harassment, social ostracisation, abuse, and exploitation of various types. In a landmark judgment, the Supreme Court of India created the "third gender" status for transgenders and intersexed individuals with specific civil rights accorded to them in various arenas of social life.

- The SC asked the Centre to treat transgender as socially and economically backward.
- The apex court said that transgenders will be allowed admission in educational institutions and given employment on the basis that they belonged to the third gender category [14]

The analysis of international precedents on transgender rights delineates two prominent models for legal recognition of their gender identity and for obtaining sexual reassignment surgery to those who need it.

Gender Dysphoria/Diagnosis Model (BASED ON WPATH)

In this model patients must receive approval from medical professionals to undergo surgery or have changes to their ID documentation after diagnosis of gender identity disorder (GID) or gender dysphoria.

Self-Identification Model (BASED ON YOGYAKARTA PRINCIPLES)

A self-identification model of gender recognition followed in India, sees right to self-determination of one's own gender is a fundamental right for all people and are not required to be diagnosed with gender dysphoria or gender identity disorder and have the right to access both hormonal treatment and surgery. Individuals over the age of eighteen years merely have to submit to the concerned government department a request to alter their birth certificate to reflect the self-determined gender [14]

Barr Body and Competitive Sports

Indian sports have been shaken by gender test controversies in recent years. Shanthi Soundarajan, female athlete who won the silver medal in 800 meters- at 2006 Asian games failed the gender verification test and was stripped of her medal. It was reported that she had AIS (androgen insensitivity syndrome, 46 XY with intersex), presenting as a phenotypic female adult owing to failure of androgen receptors [15]

Gender verification test for all female athletes began in response to suspicions that some countries sent men who masqueraded as women to gain competitive advantage in certain Olympics events. There was probably ambiguity of the external genitalia in most of these cases, possibly as a result of male pseudohermaphroditism [16]

The International Association of Athletic Federation (IAAF) made it mandatory for all female athletes to undergo genital examination by gynecologists. This outrageous practice was humiliating for the athletes and the International Olympic committee (IOC) substituted this test with a buccal smear test to test for Barr bodies. But even this test was not of much help in resolving controversial issues, as it was found that some people exhibited some sort of intersex conditions, where there existed phenotypic females with male sex chromatin patterns eg; AIS, XY gonadal dysgenesis, etc [17]

It was argued that such players have no athletic advantage as a result of their congenital abnormality and should not be barred from competitions. Sex chromatin testing does unfairly exclude many genuine athletes. Laboratory tests infact still fail to address and conclusively resolve the issue. Therefore the IAAF did a reappraisal and reached the conclusion that gender verification is not needed at all and as such screening for gender is no longer required at IAAF competitions since 2009 [16]

Nevertheless the IOC has promulgated a new rule according to which women who test in the male range for testosterone and whose bodies respond to this hormone, may not be eligible to compete as females. Such precautions are set to level the playing field preventing people who identify as women but have an unfair male like advantage [15]

More and more experts are now reaching the consensus that sex determination test of players must take in to account all aspects such as chromosome, genitals, gonads and hormones in order to reach unambiguous and undisputed decision.

Under the current policy normal XX females with an naturally high testosterone due to practice could be declared ineligible to compete as woman and the same current criteria would however favour players with XY having AIS would be deemed to be women for the purpose of sports as they exhibit low levels of testosterone [15]

Current Role of Cytogenetics and Karyotyping

The determination of the nuclear sex, a relatively easy procedure, is an important tool in investigating these developmental abnormalities if its usefulness and limitations are clearly recognized. Now that full chromosome analysis is routine, it has become apparent that there exists a possibility of both false-positive and false-negative sex chromatin patterns; therefore the pattern does not always reflect the true cytogenetic state of the individual. It cannot indicate the autosomal constitution of the individual, and it does not reflect the sex chromosome status, for it tells us nothing about the Y chromosome, and structural abnormalities of the X chromosome are not evident with routine methods. The sex chromatin test is an aid to the cytogeneticist in interpreting the chromosome analysis. 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. Karyotyping is especially essential in certain conditions where a phenotypic female without sex chromosome may have 46 XY complement and the presence of Y chromosome cause a substantial role of gonadal malignancy [18]. Such female should have prophylactic gonadectomy. Again, individual with 47 XXY who may appear like normal phenotype males in many situations are known to have criminal tendencies as observed in studies. Such individuals in the transgender population can also be screened for the additional X chromosome by mean of buccal smear test, validating the need for karyotyping.

Conclusion

In the Indian scenario, assigning gender is not easy, because most patients with ambiguous genitalia may end up as females. The problem is further complicated by the timing of presentation as it may be noticed at birth or noticed at puberty. Both need a thorough diagnostic work-up before assigning gender. The latter may have another difficulty in that the child may have been raised as a male, but eventually be assigned female sex. Ideally, a team of health care professionals with expertise in intersex should work together to understand and treat the child with intersex and to understand, counsel, and support the entire family.

Children with intersex disorders and/ or with abnormal sex chromosomes are at an increased risk for development of malignancy, particularly in their gonads. Clearly, intersex is a complex issue. Even after waiting for months to announce the birth of a child to relatives, parents are unclear if it is a girl or a boy. They are disturbed by the fact that if it is a boy his sexuality may be inadequate and if it's a girl she may not menstruate or procreate.

On the principle that a sound basis for good management of a medical problem is provided by knowledge of its biological background, the pathogenesis of anomalous sex has to be studied at its fundamental level, using the nuclear sex.

The determination of nuclear sex is an easy procedure and can be an important tool in investigating these developmental abnormalities. Sex determination using Barr bodies in buccal scrapes is a simple method providing up to 95-98% accuracy; this makes it significant accessory to other methods of sex determination. In borderline cases, it is necessary to utilize other, more elaborate methods. It can be used as a guide to karyotyping. It is essential to have such a test for large screening purposes, as it is simple, easy, cost effective, non-time consuming and non-invasive procedure.

Since the test involved does not invade the privacy, and does not violate the human right of these individuals in any way, it can be advocated to the policy makers in appropriate platform. In a country like India where assigning gender to transgender has been made following self identification model, as opposed to the gender dysphoria/ diagnosis model, it is prudent to use this nuclear sex screening tool for isolating those intersexed individuals who are at additional risk for certain malignancies or those in whom aggressive behavior or altered mental health predilection is evidenced, which would otherwise go unnoticed in the absence of a diagnosis model.

References

1. Reddy DS, Sherlin HJ, Ramani P, Prakash PA. Determination of sex by exfoliative cytology using acridine orange confocal microscopy: A short study. J Forensic Dent Sci 2012;4:66-9.
2. Stavrianos C, Methods for human identification in Forensic Dentistry: A Review, The Internet Journal of Forensic Science. 2008; 4 (1).
3. Tushar Mittal, K. Muralidhar Saralaya , Ajee Kuruvilla, Chandrayya Achary. Sex determination from buccal mucosa scrapes. Int J Legal Med (2009) 123:437–440.
4. Stanley M Gartler, X-Chromosome Inactivation. Encyclopedia of Life Sciences / & 2001 Nature Publishing Group.
5. Campbell N, Chromosomal basis of inheritance,. Biology. 5th ed, 1993.
6. Fiona Alice Miller. 'Your true and proper gender': the Barr body as a good enough science of sex. Stud. Hist. Phil. Biol. & Biomed. Sci. 2006; 37:459–483.
7. Basavaraj P Bommanahalli, Chandrashekar M, Shashikala P, Vijayakumar B Jatti. Reliability of Davidson body to determine the nuclear sex of the individual: Interobserver variability between pathologists. Indian J Forensic Med Toxicol 2011;5:11-13.
8. Sean cahill, Transgender equality- A handbook for activist and policy makers.
9. Patricia A. Jacobs, Sex Chromosome Abnormalities in the Male, Res Medica, Spring 1966, Volume 5, Number 2.
10. Ujit.,intersexed conditions- a review, Journal 9 issue 130,1732 7/20/2015.
11. Jyotsna kirtane, Ethics in intersex disorder. Issues in Medical Ethics, VIII (2), April-June 2000.
12. Dreger, "Ambiguous Sex"--or Ambivalent Medicine?; The Hastings Center May/Jun 1998.
13. Silva K S H, Usefulness of a buccal smear in the initial assessment of a baby with a disorder of sex development. Sri Lanka Journal of Child Health, 2013; 42: 189-191.
14. Venkatesan Chakrapani, Legal recognition of gender identity of trans people in India. Alternative law forum (ALF) Bangalore, 2012.
15. Arvind Mishra. Gender imbroglio in sports. Scientific reports 2013.
16. Jagadeesh N. Sex verification tests: ethical, legal and social aspects. Indian Journal of medical ethics. 2013. Vol 10, No 1.
17. Vanessa, Testing sex and gender in sports; reinventing, reimagining and reconstructing histories. Endeavour. Dec 2010; 34(4): 157–163.
18. Mary F. lyon, Sex Chromatin and Gene Action in the Mammalian X-Chromosome, Received Aug. 21, 1961.