CHROMOSOME ANALYSIS FROM LYMPHOCYTE CULTURE IN SEXUAL AMBIGUITY PATIENT

Ni Wajan Tirthaningsih, Susy Kristiani
Department of Anatomy and Histology
Airlangga University School of Medicine
Surabaya

ABSTRACT

At least three parameters are required for sex assignment, namely: chromosomal sex (karyotype), gonadal sex and genitalia sex. Sexual ambiguity will occur if one of those parameters is not in accordance to the other, which in turn gives rise to many problems, especially social problems. Chromosome analysis had been done to 40 patients with sexual ambiguity, who came to Clinical Genetic Unit of Airlangga University School of Medicine, Dr. Soetomo Hospital Surabaya from January 2004 till May 2005. The results are as follows: 29 patients (72.5 %) with a 46,XY karyotype (which were actually male pseudohermaphroditisms); 9 patients (22.5 %) with a 46,XX karyotype (which were actually female pseudohermaphroditisms); 2 patients (5 %) with a 46,XX karyotype (which were actually true hermaphrodites).

Keywords*: sexual ambiguity, karyotype, pseudohermaphroditisms*

Correspondence: Ni Wajan Tirthaningsih, Department of Anatomy and Histology, Airlangga University School of Medicine, Jl Mayjen Mustopo 47, Surabaya, phone: 62-31-5020251 ext. 148

INTRODUCTION

Generally, as an infant is delivered, the first question that comes in mind from the parents is: Is it going to be a boy or a girl? So, when this doubtful situation occurs, it leads to anxiety and deeper apprehension. Therefore, a quick and accurate effort to recognize the sex of an infant is greatly needed, so that parents can determine a choice without a doubt to raise the child either as a boy or a girl, before any circumstances emerge before making wrong decision on the child's sex.

Besides, sexual ambiguity includes as one of the social emergencies because it would be difficult and complicated for parents to have the child's birth certificate processed as the sex is ambiguous. Not only that, teenagers will suffer psychological impact since they are not prepared to face problems that are related to sexual ambiguity, it is also not possible for them to submit to depression and drug abuse.

Sex differentiation disturbance causes sexual ambiguity that is divided to three groups and regarded as male pseudohermaphroditism, female pseudoherma-phroditism and true pseudohermaphroditisms (Connor 1997; Emery 1990; Lewis 2003; Simpson 1990). Sexual ambiguity problem has become a major issue in the world of surgery. Before a surgery is conducted, a parameter and consideration to determine which sex to live should be acknowledged first, so that no complications should occur in the future.

A chromosome analysis had been conducted in a sexual ambiguity patient at Genetic Clinic Unit of Airlangga University School of Medicine, Dr. Soetomo Hospital Surabaya, where the results are anticipated as an example and consideration in deciding to which steps to manage the sexual ambiguity patient.

Sexual Ambiguity

Sex differentiation disorder causes sexual ambiguity that is divided into three groups and regarded as male pseudohermaphroditism, female pseudoherma-phroditism and true pseudohermaphroditisms (Connor 1997; Emery 1990; Lewis 2003; Simpson 1990).

Male pseudohermaphroditism (male intersexuality) is a state where an individual with 46 karyotype,XY or with Y chromosome has a pair of testis and the external genitalia undergoes inadequate masculinization. Male pseudohermaphroditism is commonly found in hypospadia or micropenis together with cryptochidism, either as a disorder or part of a syndrome. The most extreme appearance of this form is Testicular Feminization Syndrome (Complete androgen intersensitivity syndrome), where the external genitalia appears as a normal female even at the day the infant is delivered, thus showing no difficulty to determine the sex. Problems occur after puberty and after getting married because of primary amenorrhea and infertility.

Female pseudohermaphroditism is a state where there is a pair of normal ovaries, karyotype 46,XX and external genitalia virilization. The best example is Congenitalia Virilization Adrenal Hyperplasia, which is caused by enzyme deficiency that involves cortisol metabolism or 21-hydroxylase enzyme due to autosomal recessive disorder. This is the most important form of external genitalia ambiguity that is caused by mineral corticoid deficiency complication. A disorder of cortisol deficiency causes ACTH secretion, adrenal cortical hyperplasia and excessive production of adrenal androgen. The degree of virilization varies from clitoral hypertrophy to labioscrotal fusion, together with complete urethral in a penis. An advance stage is where a woman appears as a man with adescensus testis. External genitalia virilization in woman occurs due to exogenous androgen that is given to pregnant mother or androgen-produced tumor during pregnancy (virilization tumor) (Capelle 1972, 1981).

True pseudohermaphroditisms is a state where there are ovarian and testicular tissues and often have one or more ovotestis tissues. Chromosome 46,XX is commonly found (Ramsay 1988; Simpson 1990; Skordis 1987). Chromosome 46,XX/46,XY or 46,XY can also be found. Such condition is generally related to sexual ambiguity and there are both male and female internal genitalia.

Parameter to Determine Sex

An individual sex is determined mainly by a TDF gene at the short arm of chromosome Y (Yp). The effect of this gene on gonadal system (that has not been differentiated) will be resulted differentiation into testis that will produce testosterone that causes Muller duct regression and development of Wolf duct resulting in the appearance of male genitalia.

Therefore, to determine the sex, three parameters should be ascertained, which are chromosomal sex, gonadal sex and sexes based on external genitalia appearance and sex identity (Connor 1997; Lewis 2003; Thompson 1991; Watson 2004). These parameters should be coherent to each, otherwise, sexual ambiguity will occur.

Chromosomal sex is determined by existing chromosomal sex, where karyotype 46,XX is for normal woman and 46,XY for normal man. However, there are cases where it is difficult to determine sex, such as mosaicism (45,X/46,XY and 46,XX/46XY), 46,XX male and testicular feminization syndrome (46,XY). Gonadal sex determination is based on gonad histological characteristics, where there are follicles to ovaries and seminiferous tubule to the testis. Gonadal biopsy is needed to confirm the diagnosis and manage hermaphrodite patients. Genital sex is the most common way to determine sex. This method is used as guidance in nurturing a child, where it is easily noticeable and questionable when sexual ambiguity occurs. Sex identity is a personality and emotional trait that arises before hormonal effects or significant physical appearance. This trait is inherited by the parents through the name given, clothes model, hair cut, toys and others. Sex identity does not exist the moment of delivery, but it becomes more apparent along with the course of time. This condition should be handled seriously, either by counselling or other methods to determine a child's sex.

MATERIALS AND METHODS

This was a descriptive observational study. Samples consisted of 40 sexual ambiguity patients who came to the Genetic Clinic Unit Airlangga University School of Medicine, dr. Soetomo Hospital Surabaya and blood were taken from their veins as much as one cc with anti-coagulant Natrium heparin. Even though Testicular Feminization Syndrome is considered as Male pseudohermaphroditism, it was not included in this study because the patients did not complain sexual ambiguity, but with primary amenorrhea or infertility.

About 0.3 ml was inserted into a sterile culture tube and was cultured with a Whole Blood Microculture technique (Benn 1986). After being incubated in a CO_2 incubator at the temperature of 37^0c, cells will stop dividing at metaphase stage with the addition of colcemide 0.2 mg/ml. Subsequently, a hypotonic substance was given (KCl 0.075 M) that caused the cells to swell and each of the chromosome was separated. After that phase, a fixation was done with substances of acid acetate:methanol (1:3) (Benn 1986).

Afterwards, six slides were prepared for each patient by dropping substances on object glasses, dried at the temperature of 60^0c for two to three days, and later were stained with G-comparison technique (Benn 1986). The slides were observed under microscope at magnification of 1000 to 1500 with emersion oil and the total and structures of chromosomes were analysed to determine the karyotypes. An experienced genetic expert was asked to analyse the chromosome.

RESULTS

Results of the chromosome analysis from the samples who came to the unit from January 2004 to May 2005 can be seen in the Table 1.

Table 1. Karyotype sexual ambiguity patients

Clinical	Karyotype	Total	%
Male pseudohermaphroditism	46,XY	29	72.5
Female pseudohermaphroditism	46,XX	9	22.5
True hermaphroditism	46,XX	2	5

DISCUSSION

From the samples, most of the patients, (72.5%) were male pseudohermaphroditism, came with micropenis or hypospadia that seems to be an isolated disorder, with karyotype 46,XY. Authors, Garver et al. (1986) and Simpson (1990) also found the same findings. From the results of this observation, cases with karyotype 45,X/46,XY or 45,X/47,XYY that can cause male pseudohermaphroditism, had not been found. It is possible that the size of the samples was too small. Mosaicism chromosomal disorders such as 45,X / 46XY / 47,XYY, even though were very rare to find, still can cause male pseudohermaphroditism, although not frequently diagnosed. Table 1 shows, female pseudohermaphroditism patients have karyotype 46,XX because most of the cases are due to genetic disorders (Garver et al. 1986). However, mosaicism chromosomal disorders can also cause female pseudohermaphroditism such as 45,X / 46,XY, even though it is very rare to find. Five percent of the sexual ambiguity cases are true hermaphroditism with karyotype 46,XX, the most common cases (Simpson 1990). Other karyotypes that accompanied true hermaphroditism such as 46,XX / 46,XY or 46,XY (Garver 1986; Simpson 1990), could not be found in this research. This is perhaps due to the size of the samples that is too small and very rare to be found.

CONCLUSION

From this study, it is concluded that external genitalia appearance that becomes the base of clinical diagnosis, often does not coherent to chromosomal sex (karyotype). Every case with sexual ambiguity should notice at least three parameters, the genital sex, chromosomal sex and if possible, gonadal sex to determine a child's sex Fast and accurate sex determination is greatly needed in the ways to nurture a child. It also helps to evaluate issues that are related to these disorders (such as body fluid and electrolyte disturbance on female infant suffering from virilization/female pseudohermaphroditism). Additionally, a specific medication and surgical treatment can be planned as early as possible. An advanced research should be conducted by adding total samples and intensifying chromosomal examinations in order to receive more accurate pictures of chromosomal disorders that underlie the sexual ambiguity.

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