

# Role of Cytogenetics in Gender Confirmation

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## ABSTRACT

**Objective:** To determine the Karyotype of referred cases of Gender Ambiguity through Cytogenetic study.

**Patients and Methods:** This cross-sectional study was carried out at Islamabad Diagnostic Centre, Islamabad from April 2010 to June 2017. A total of 72 cases of Gender Ambiguity aged 1day to 35 years were referred to IDC for chromosomal analysis and karyotyping during above mentioned period. Out of these 72 cases, 37 presented with somewhat male-like external genitalia and 35 with more of female-like external genitalia but clear-cut gender differentiation was not possible on external physical examination. Hence Cytogenetics was performed on peripheral blood to confirm their chromosomal makeup.

**Results:** Out of the total 72 cases, 12 cases that were suspected to be female on external physical examination, turned out to have male karyotype (46, XY). Conversely 7 cases that were suspected to be male on external physical examination, turned to have female karyotype (46, XX). Twenty seven cases with male-like clinical gender were confirmed to have male karyotype (46, XY) and 22 cases with female-like clinical gender were confirmed to have female karyotype (46,XX).

**Conclusion:** Cytogenetic study is essential in confirming the chromosomal makeup of individuals born with ambiguous gender. It should be carried out as early as possible in life for appropriate brought up and social adjustment of the affected individual.

**Keywords:** Ambiguous Genitalia, Cytogenetics, Gender Confirmation, Karyotype

### Author's Contribution

<sup>1</sup> Data analysis, interpretation and manuscript writing, Active participation in data collection

<sup>2</sup> Conception, synthesis, planning of research and manuscript writing

<sup>3</sup> Interpretation and discussion

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## Introduction

It is a great blessing of God Almighty to be born normal and with a clear gender either male or female. Indeed, it is a big stress for parents if the newborn has some congenital anomaly or the gender is ambiguous due to malformed external genitalia. Such cases of gender ambiguity are not rare. Their incidence is approximately 1 per 4500 live births.<sup>1</sup> Gender differentiation is under a

complex control of specific genes on chromosomes along with hormonal regulation.<sup>2</sup> While many cases are the result of virilising effect of adrenal hormones in Congenital Adrenal Hyperplasia (CAH), a significant number is because of genetic abnormalities resulting in True Hermaphroditism, Gonadal Dysgenesis and Pseudo-hermaphroditism.<sup>3</sup> A newborn with ambiguous genitalia

requires urgent evaluation to detect life threatening conditions such as salt-losing crisis in Congenital Adrenal Hyperplasia (CAH) and also for early assignment of gender to minimize the stress of parents having a baby with unidentified sex.<sup>4</sup> A thorough physical examination of the patient along with relevant imaging tests, hormonal assay, molecular and cytogenetic assay is essential to make a correct diagnosis, so as to properly counsel the parents. Proper gender assignment should be done at the earliest so as to make use of various therapeutic options to enable the affected child to develop into a psychosocially stable individual.<sup>5,6</sup> Initially, both male and female embryos have two pairs of genital ducts: mesonephric (Wolffian) ducts and paramesonephric (Mullerian) ducts.<sup>7</sup> Sex differentiation is a complex process involving many genes. The key factor to sex differentiation is Y chromosome which contains SRY gene (sex determining region on Y) on its short arm (Yp11). Its presence determines the fate and development of sexual organs. Under the influence of SRY gene male development occurs and its absence leads to female development.<sup>8</sup> Some individuals carry a Y chromosome but are phenotypically female (46 XY). This could be due to loss of SRY gene. Some patients are phenotypically male but have female Karyotype (46, XX). This is due to translocation of SRY gene on an X chromosome or some autosome.<sup>3</sup> The genetic sex of embryo is established at the time of fertilization whereas phenotypic sex development occurs during the period of organogenesis when the gonads develop. Apart from sex specific genes on X and Y chromosomes, other autosomal genes also play a role in sex determination.<sup>9</sup>

Patients of gender ambiguity are evaluated with Cytogenetic analysis for detecting X and Y chromosomes and determining the exact Karyotype of the individual. Ideally the baby born with ambiguous genitalia should be managed in a tertiary care setup by a multidisciplinary team including pediatric endocrinologist and urologist, geneticist, gynecologists and clinical psychologist with access to specialist lab and radiology services.<sup>10</sup>

## Patients and Methods

A total of 72 cases of gender ambiguity, aged 1 day to 35 years, were referred to Islamabad Diagnostic Centre, Islamabad from April 2010 to June 2017 for Cytogenetic

Analysis and Karyotyping. Out of these 72 cases, 37 presented with somewhat male-like external genitalia and 35 with more of female-like external genitalia but clear cut gender differentiation was not possible on external physical examination. Hence cytogenetics was performed on peripheral blood to confirm their chromosomal makeup. In all cases heparinized peripheral blood samples were subjected to 72 hours' culture at 37<sup>o</sup> C on PB- Max medium with Phytohaemagglutinin (PHA) as a stimulant for lymphocytic cells division induction and mitosis. After colchicine treatment, harvesting was done as per standard protocol. Upto 20 metaphases were analyzed microscopically in each case after Giemsa Trypsin banding.

## Results

The study included 72 cases of ambiguous gender referred for Cytogenetic analysis and Karyotyping to confirm chromosomal makeup. Among these 72 cases of ambiguous genitalia 46 (63.9%) cases were aged 1 day to 3 years while 08 (11.1%) cases belonged both to 4 to 7 years and 8 to 11 years' age groups. Four (5.6%) cases were between 12 to 15 years and 6 (8.3%) belonged to 16 to 35 years of age. (Table 1)

Variable	No. of Cases	Percentage
1day to 3 years	46	63.9
4 to 7 years	08	11.1
8 to 11 years	08	11.1
12 to 15years	04	5.6
16 years and above	06	8.3

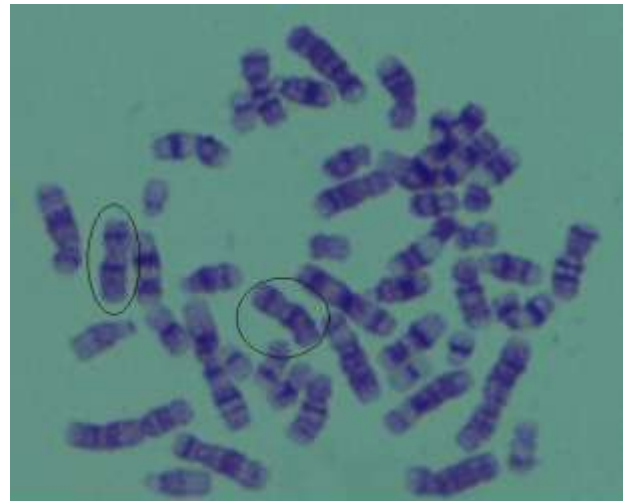
On detailed chromosomal analysis of the 72 cases (Table 2), interestingly 12 cases with provisional gender of female on physical examination turned out to be Cytogenetically Male with 46, XY Karyotype while 7 cases with provisional gender of male turned out to be Cytogenetically Female with 46, XX Karyotype. Twenty-seven cases who were suspected to have male gender on clinical examination were confirmed to have 46, XY Male Karyotype. Similarly, 22 cases with clinical suspicion of female gender were confirmed to have 46, XX Female Karyotype. Additional Karyotypic findings were also noted in 4 cases which included: 45, XO/46, XY (p-), 46, XY with

small Y chromosome but intact 'p' and 'q' arms, 47, XYY /45, XO mosaic and 45, XO Turner's Syndrome.

Table2. A Summary of findings in 72 Cases		
Predominant Physical Presentation	Karyotype	No of cases
Phenotypically Males (n=37)	46, XY Male Karyotype	27
	46, XX Female Karyotype	07
	45, XO/46, XY (p-) Absent/Small Y chromosome with deleted short arm	01
	46, XY with unusually small Y chromosome but intact short and long arms.	01
	47, XYY/45XO	01
Phenotypically Females (n=35)	46, XX Female Karyotype	22
	46, XY Male Karyotype	12
	45, XO Turner's Syndrome	01



Figure 1: 46, XY Male Karyotype



46,XX Metaphase Female Karyotype

Figure 2: 46,XX Metaphase Female Karyotype

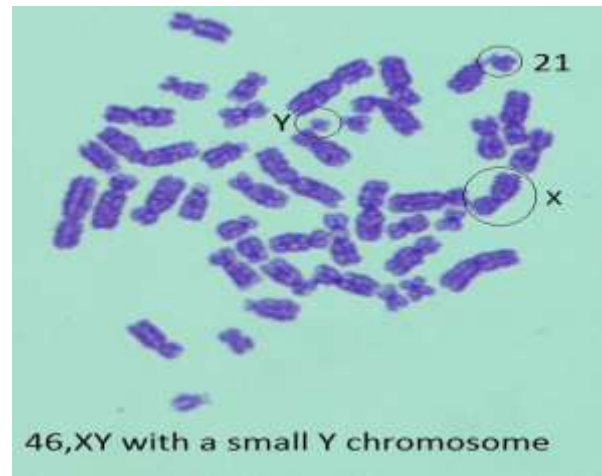


Figure 3: 46,XY with a small chromosome

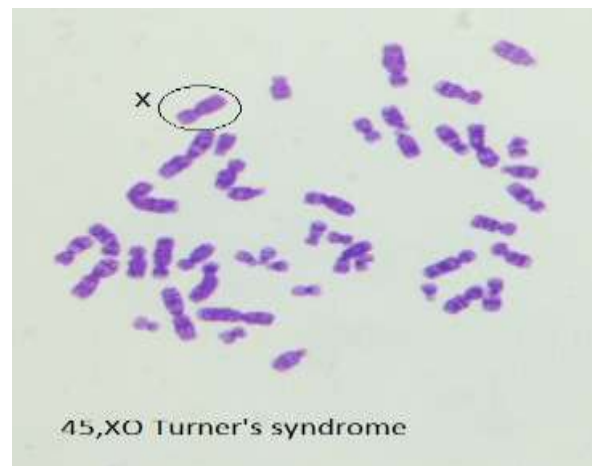
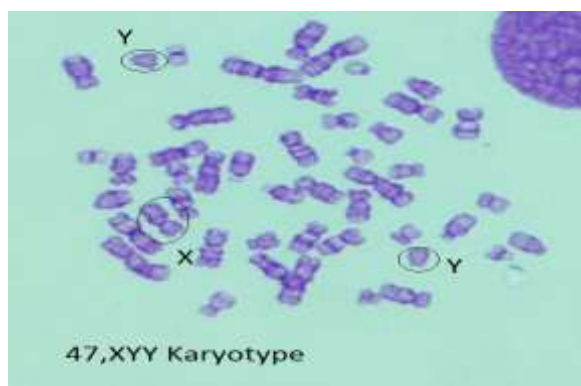
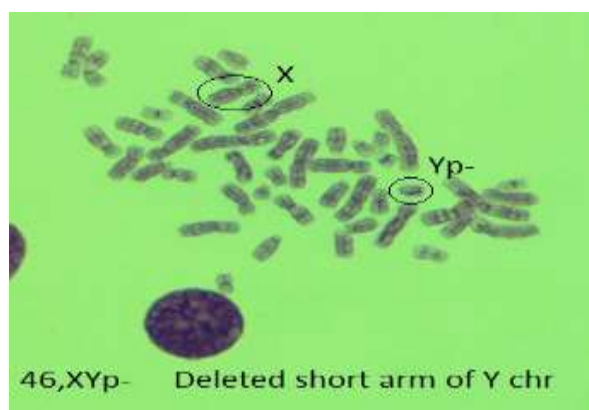


Figure 4: 45,XO Turner's syndrome



**Figure 5: 47,XYY Karyotype**



**Figure 6: 46,XYp- Deleted short arm of Y chromosome**

## Discussion

Disorders of sex development (DSD), formerly termed “intersex” conditions, arise from numerous causes. Congenital adrenal hyperplasia (CAH), secondary to 21-hydroxylase deficiency is the most common cause of DSD. Sex chromosome disorders, including sex chromosome mosaicism, are the second most common cause of DSD.<sup>11</sup> The main purpose of our study was the Cytogenetic evaluation of patients with ambiguous genitalia. Determination of chromosomal karyotype is very important for the appropriate counseling and effective management of patients with gender ambiguity.<sup>12</sup> Sex determination is based upon presence or absence of Y chromosome. As a general rule, XY embryos are males whereas XX embryos are females. However, many a times, hormonal influences during gestational period results in babies born with ambiguous external genitalia. This leads to social stresses for the parents as to whether to declare the newborn as a girl or a boy. Many worried

parents seek consultation of pediatricians for this purpose who advise imaging, hormonal and cytogenetic testing to confirm the gender of these babies born with ambiguous external genitalia. Sometimes the testing is quite delayed due to lack of awareness on the part of the individuals concerned. Hence early physical/clinical examination, hormonal and cytogenetic analysis is essential in presumptive etiological diagnosis in cases of disorders of sexual development (DSD).<sup>13</sup>

In our study, out of 72 cases of ambiguous genitalia referred to our center, 46 (63.9%) cases were of 1 day to 3 years of age while 08 (11.1%) cases were of 4 to 7 years of age. This is comparable to the report by Pandit et al<sup>3</sup> in which among 50 cases of ambiguous genitalia 27 (54%) cases were of 1 day to 3 years of age and 09 (18%) were of 4 to 7 years of age. In both studies, around 3 quarter of the cases were less than 7 years of age. In our study 12 cases who were thought to be physically female turned out to be cytogenetically male with 46XY Karyotype while 7 cases that were physically suspected to be male conversely turned out to be cytogenetically female having 46,XX Karyotype. This further signifies the importance of Cytogenetic studies early on in life so that exact chromosomal /genetic gender is confirmed for initiation of any therapy needed and appropriate brought up of the affected kid. In another study 156 cases with varied abnormalities of sexual development had cytogenetic analysis between 1991 till 2001. Out of these 68 had been raised as males (43.6%) and 88 as females (56.4%). On chromosomal analysis 4 out of the 68 raised as males were found to have 46 XX karyotype.<sup>14</sup>

In our study, out of the 72 cases of ambiguous gender, 27 cases who presented with somewhat male-like ambiguous external genitalia were confirmed to have Male (46, XY) Karyotype on cytogenetic study. Similarly, 22 cases presenting with female-like ambiguous external genitalia were confirmed to have Female (46, XX) Karyotype on cytogenetics. In another study, chromosomal 40 cases with ambiguous genitalia was done.<sup>15</sup> Out of these 20 were reared as males and 20 as females. While 8 cases were below the age of one year, 18 (45%) were in the age bracket of 1-5 years. In these 40 cases, 21 had 46 XY karyotype, 13 had 46, XX karyotype, 2 cases showed true hermaphroditism i.e. 46,



XX/46 XY karyotype while 4 cases showed mosaic pattern with 46, XO/46, XY karyotype.

### Conclusion

Cytogenetic study is a vital tool in confirming the gender of patients born with ambiguous external genitalia. The earlier this study is carried out, the better it is for appropriate counseling of parents and initiation of any therapy needed. Delay in determining the gender of the individual leads not only to social stresses for the parents but also hampers the appropriate brought up of the individual with associated psycho-social effects and adjustment issues in later life. Hence there is need to create awareness amongst the masses about the availability of genetic testing to know the exact gender of the individuals born with ambiguous external genitalia early on in their life.

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