

## **Cytogenetic Analysis of Children with Ambiguous Genitalia in Mosul**

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### **ABSTRACT**

The present study aim to investigate the causes and characteristics of ambiguous genitalia in newborn who were referred to cytogenetic laboratory. Cytogenetic analysis was performed for each case. All cases were analyzed by chromosomal study using GTG karyotype. All patients after cytogenetic analysis revealed 46, XX and 46, XY Karyotype. One individual carry a Y chromosome but phenotypically female and one case have a female karyotype but are phenotypically male. The cytogenetic analysis of children with suspected chromosomal aberrations is important to uncover the contribution of chromosomal disorder in genesis of sexual ambiguity and congenital malformation in children and prevent further potentially unpleasant investigation being undertaken

**Keywords:** ambiguous genitalia, Cytogenetic analysis.

46XX , 46XY

## INTRODUCTION

Sexual ambiguity is a complex and often confusing medical problem in newborn. Determining the appropriate sex in these patients is an urgent matter, particularly in congenital adrenal hyperplasia (CAH) (Frimberger and Gearhart, 2005). Ambiguous genitalia in the newborn and children need immediate and rational management and assignment of sex for rearing should be guided by the etiology of the genital malformation (Sultan *et al.*, 2002). The importance of the Y chromosome in male determination has been well established since the 1950's (Mendes *et al.*, 1999). The main factor influencing the sex determination of an embryo is the genetic sex determined by the presence or absence of the Y chromosome (Ergun-Longmire *et al.*, 2005). As a general rule, XY embryos become males and XX embryos become females. However, some individuals carry a Y chromosome but are phenotypically female (46,XY females) or have a female karyotype but are phenotypically male (46,XX males). 46,XX maleness is a rare sex reversal syndrome affecting 1 in 20,000 newborn males. Molecular analysis of sex-reversed patients led to the discovery of the SRY gene (Berta *et al.*, 1990). Human sexual differentiation is a highly complex process under the control of multiple genes and hormones. Abnormalities in normal sexual differentiation are relatively common and occur in approximately 1 per 4500 live birth. Human males with a 46,XX karyotype were first described in 1964 by three different groups of investigators (Sambrook and Fritsch, 1989). The present study aim to analyze the chromosomes of children those suffer from ambiguous genitalia in children during (2009-2011) in Mosul Iraq.

## MATERIALS AND METHODS

A short term culture of peripheral blood lymphocytes (5ml) was collected in a heparinized syringe in the cytogenetic laboratory and culture was done by using 5 ml of (RPMI-1640) media, 1 ml fetal calf serum and 0.1 ml of Phytohemagglutinin. The culture vials were kept in an incubator for 72 hours at 37C°. colcemid was added to arrest cell division at metaphase stage. Harvesting was done by adding 10 ml of KCL (0.56 %) for 20 minutes. The cells were fixed with fixative 3:1 (methanol: acetic acid) after discarding the supernatant. The slides were prepared and stained with 4% Giemsa stain. Twenty well spread metaphases were screened under the light microscope X100 magnification. Microscopic examination cells were done after standard trypsin G-banding (GTG) (Yassen, 1990) was prepared by arranging homologous chromosomes in pairs according to international system for cytogenetic nomenclature (ISCN, 1995).

## RESULT

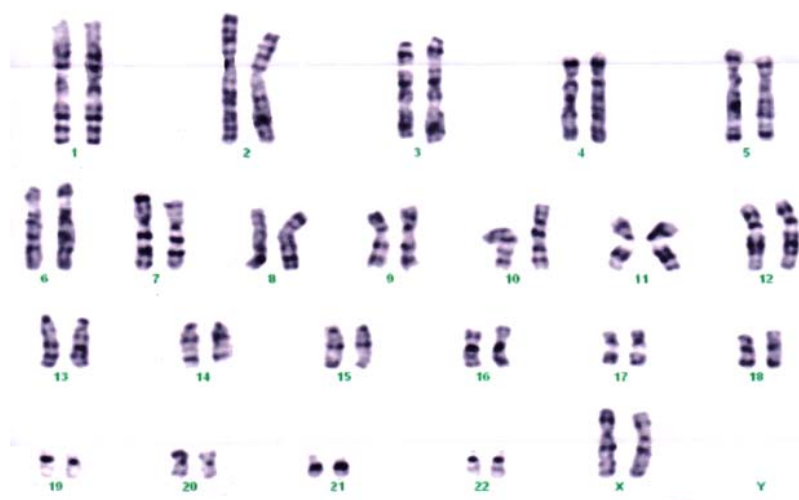
This study was conducted in 20 children with age ranging from ten days to one year. All patients presented 46XX and 46XY karyotype at cytogenetic analysis. Clinical and cytogenetic finding of the patient are shown in Table (1). While in all patient were detected 46,XX and 46, XY Karyotype by cytogenetic analysis is one individual carries a Y chromosome but phenotypically female or one of cases has a female karyotype but are phenotypically male such as in Fig. (1,2).

**Table 1: Clinical and cytogenetic findings of the patients.**

Case	Age	Social sex	External genitalia	Cytogenetic Diagnosis
1	20 day	M	Ambiguous	46,XY
2*	10 day	F	Ambiguous	46XY
3	28 day	F	Ambiguous	46XX
4	1 year	F	Ambiguous	46XX
5	50 day	F	Ambiguous	46XX
6	15 day	M	Ambiguous	46XY
7	44 day	M	Ambiguous	46XY
8	90 day	F	Ambiguous	46XX
9	1 year	M	Ambiguous	46XY
10	22 day	M	Ambiguous	46XY
11	30 day	F	Ambiguous	46XX
12	123 day	F	Ambiguous	46XX
13*	1 year	M	Ambiguous	46XX
14	40 day	F	Ambiguous	46XX
15	20 day	F	Ambiguous	46XX
16	1 year	F	Ambiguous	46XX
17	23 day	F	Ambiguous	46XX
18	39 day	M	Ambiguous	46XY
19	26 day	F	Ambiguous	46XX
20	12 day	F	Ambiguous	46XX

F=female, M= male

\*= case of ambiguous



**Fig. 1: Chromosomal analysis of ambiguous genitalia of female genotype but male phenotype.**



**Fig. 2: Chromosomal analysis of ambiguous genitalia of male genotype but female phenotype.**

## DISCUSSION

In this research was found from twenty cases are ambiguous genitalia two cases, were detected 46,XX and 46,XY Karyotype by cytogenetic analysis is one individual carries a Y chromosome but phenotypically female and one of case have a female karyotype but are phenotypically male. Our study consistent with many studies. Human males with a 46,XX karyotype were first described in 1964 (Court-Brown *et al.*, 1964 ; Rajender *et al.*, 2006). The frequency of this syndrome has been estimated to be 1 in 20,000-25,000 newborn males, although there are considerable geographic variations molecular analyses have demonstrated that approximately 90% of patients with 46, XXkaryotype carry a variable amount of Y material due to a Y-to-X interchange originated by an illegitimate recombination during paternal meiosis (Perez-Palacios and Kofman, 1994). In (Kusz *et al.*, 1999) demonstrated that in XX males with Y-to-X translocations, preferential inactivation of the Y-bearing X chromosome could be the major mechanism causing a sexually ambiguous phenotype (Rajender *et al.*, 2006). The origin of male phenotype in XX males could be the result of at least three different mechanisms: 1) translocation of Y sequences, including the SRY gene, to an X chromosome or to an autosome; 2) a mutation in a yet unknown X-linked or autosomal gene in the testis-determining pathway, and 3) cryptic Y chromosome mosaicism (Zenteno-Ruiz *et al.*, 2001). XX males can be classified as Y-positive or Y-negative depending on the presence or absence of Y-derived specific sequences.

In conclusion, the cytogenetic study at chromosomal level for new born with ambiguous genitalia need more investigation to supporting the result using PCR analysis of the SRY gene provides information about the presence of a Y chromosome within 1 day (Rajender *et al.*, 2006). While we recommended the physician managing these families could minimize the trauma of having a child with unidentified sex by providing appropriate genetic counseling so that the parents can make an early decision. Prenatal DNA testing in at-risk families should be considered and appropriate therapy offered to minimize or prevent

genital ambiguity. The birth of a newborn with ambiguous genitalia frequently comes as a surprise for the parents, therapist. Although some authors report that 60% of affected children are diagnosed prenatally, many parents are faced with the situation at birth (Frimberger and Gearhart, 2005). Physical examination is the key for diagnosis. Careful palpation to locate gonads at the genital folds or in the inguinal region provides the first element for diagnostic orientation. If gonads are absent, a diagnosis of female pseudohermaphroditism seems advisable; if gonads are palpated, a diagnosis of male pseudohermaphroditism is more appropriate (Rajender *et al.*, 2006).

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