

Original Research Article

Genital ambiguity: a cytogenetic evaluation of gender

K. S. Lekha^{1*}, V. Bhagyam², P. D. Varghese³, M. Manju²

¹Department of Anatomy, Government Medical College Thrissur, Kerala, India

²Department of Anatomy, Government Medical College Kozhikode, Kerala, India

³Department of Anatomy, Government Medical College Alappuzha, Kerala, India

Received: 15 December 2020

Accepted: 31 December 2020

*Correspondence:

Dr. K. S. Lekha,

E-mail: drkslekha@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Genital ambiguity is a complex genetic disorder of sexual differentiation into male or female. The purpose of the present study is to correlate the sex of rearing with the genetic sex and to find out the prevalence of chromosomal anomalies in patients with ambiguous genitalia. The findings can help in proper diagnosis, genetic counselling, and the reassignment of sex, if necessary.

Methods: In this cross-sectional study, 22 patients from north Kerala, ranging in age from 17 days to 17 years, were included. All cases were subjected to the following: a detailed history, physical examination, evaluation of clinical data, and cytogenetic analysis. Based on the standard protocol, peripheral blood lymphocyte culture was done. Chromosomal analysis was carried out with the help of an automated karyotyping system after G-banding of chromosomes.

Results: Out of the 22 patients with ambiguous genitalia, 12 patients were genetic females with karyotype 46, XX, and nine patients were genetic males with 46, XY karyotype. One was a rare variant of Klinefelter syndrome with karyotype 49, XXXXY. The most common diagnosis was congenital adrenal hyperplasia, followed by partial androgen insensitivity syndrome. Discrepancies between genetic sex and sex of rearing were noted in 27% of the cases.

Conclusions: This study unfolds the variable etiology of ambiguous genitalia and emphasizes the importance of karyotyping in diagnosis, proper assignment of the sex, and appropriate management of patients with genital ambiguity.

Keywords: Ambiguous genitalia, Cytogenetic study, Karyotyping

INTRODUCTION

Genitalia can be defined as ambiguous when it is not possible to categorize the gender of the child based on the outward genital appearance.¹ Genital ambiguity is a rare and complex disorder. For some newborn babies, determination of sex is difficult or impossible as the genitalia are ambiguous with anomalies that resemble that of the opposite chromosomal sex.

The birth of a child with ambiguous genitalia is an emotional crisis for the family.^{2,3} Assessment of genital ambiguity in the newborn is a psychosocial and medical

emergency as sometimes life-threatening medical conditions like salt-losing congenital adrenal hyperplasia (CAH) may be associated with ambiguous genitalia, which, if unrecognized, may lead to shock and death.³⁻⁷ A successful outcome in all cases depends on early recognition, correct assignment of sex within a short period, and effectively dealing with the family's concerns and anxiety.⁴

Human sexual differentiation is a highly complex process and is under the control of multiple genes and hormones. The sequential process of normal sexual differentiation begins with the establishment of chromosomal sex at the

time of fertilization. Chromosomal sex, in turn, determines gonadal sex which results in the development of both internal genital tract and external genitalia and thus establishes the phenotypic sex of the individual, which occurs later in development (from about 5–6 weeks of gestation). Disturbance to any step in the above sequential process during embryogenesis can affect sexual differentiation.^{8,9} Apart from sex-specific genes present on X and Y chromosomes, autosomal genes also play a dominant role in sex determination.^{2,10} Cytogenetic abnormalities of the autosomes- 1, 2, 9, 10, 12, and 22 have been reported to be associated with cases of ambiguous genitalia.¹¹

The incidence of genital ambiguity that results in the child's sex being uncertain is one per 4500 children.^{1,2,12,13} Treating a child with ambiguous genitalia is one of the most challenging diagnostic and therapeutic problems. The nomenclature 'intersex,' 'hermaphrodite' and 'pseudohermaphrodite' are unhelpful and perceived to be pejorative by some affected families. In its place, the term recommended is Disorder of Sex Development (DSD), and the karyotype is used as a prefix to define the category of DSD.¹⁴ General DSD categories are sex chromosome DSD; 46, XX DSD, 46, XY DSD; ovotesticular DSD; 46, XX testicular DSD; and 46, XY complete gonadal dysgenesis.¹⁵

Children born with ambiguous genitalia should be evaluated to rule out lethal conditions like CAH, and the gender of the child must be assigned as rapidly as possible to avoid later complications.^{13,16} Ambiguous genitalia can be due to fetal endocrinological abnormalities or defective gonadal development.¹ Such anomalies vary from mild hypospadias in males to the enlarged clitoris in females.¹¹ In most cases, a specific diagnosis can be made by genetic, endocrine, phenotypic, and chromosomal assessment.

Determination of the child's karyotype is the first investigation to be undertaken in finding out the etiology of ambiguous genitalia and it can help to guide surgical and psychosocial management as well as genetic counseling.^{7,17} Karyotyping also provides important clues about the location and nature of genes involved in sex determination and differentiation.¹¹ Only a very few studies have been conducted in patients with genital ambiguity in Kerala. To overcome the above limitation, the present study was undertaken to reveal the correlation of the genetic sex and the sex of rearing in children with ambiguous genitalia and to outline the investigations necessary. The ultimate goal is to unfold the etiology of ambiguous genitalia for the precise diagnosis and appropriate gender assignment of the affected child. It is important to note that sex does not indicate gender; sex refers to the biology of the internal and external genital structure. The role assigned by society in response to all the developmental manifestations of sex is gender identity. Early gender assignment is essential for the child to develop into a well-adjusted, psychosocially stable individual.

In this study, we present the clinical findings and cytogenetic data of a group of subjects with various degrees of sexual ambiguity.

METHODS

Subjects

We conducted a cross-sectional study. The study subjects included patients with ambiguous genitalia who were referred to the cytogenetic laboratory of the Department of Anatomy, Government Medical College Kozhikode, Kerala. Patients were referred from various departments of the medical college- mainly Obstetrics and Gynecology, Pediatrics, Endocrinology, and Plastic surgery, for chromosomal analysis; and they belonged to different parts of north Kerala. Their ages ranged from 17 days to 17 years and included mainly neonates and children. Family history was recorded for information regarding consanguinity, affected siblings in the family, and sex of rearing up of the child. Maternal history was taken with stress on exposure to androgen, virilization symptoms, or use of drugs during pregnancy. Detailed physical examination was done to examine external genitalia as the size of the phallus, labioscrotal folds, gonads, and other anomalies. Secondary sexual characters were noted as development of breast, axillary and pubic hair and change in voice, and any other abnormalities were observed. We utilized the prader staging system and tanner staging for physical examination. Prader stages I–V describe increasing virilization from a phenotypic female with mild clitoromegaly (stage I) to a phenotypic male with glandular hypospadias (stage V).^{4,9}

The findings were correlated with the clinical findings and clinical reports of ultrasonography, biochemical, and hormonal assay. The chromosomal analysis by peripheral lymphocyte culture was done in all the cases. Evaluation of clinical and laboratory reports was undertaken for each patient. This study was conducted as per the norms of the Institutional ethics committee over a period of seven years.

Inclusion criteria

Subjects with abnormal appearance of external genitalia with difficulty in assigning gender or with genitalia not typical of male or female were included in the study.

Exclusion criteria

Subjects above 20 years of age were excluded.

Also, from each patient/guardian, consent was obtained that their images and the information of the study may be discussed, presented, or published after concealing the identity, strictly maintaining the confidentiality of the patient.

Procedure

One ml of peripheral blood was collected for each patient in a heparinized syringe. Then, 0.5ml of heparinized whole blood was inoculated into labeled culture tubes containing 10ml of peripheral blood medium (Sigma / Gibco). The main components of the medium are - RPMI 1640 with L gluten, fetal bovine serum, phytohemagglutinin (PHA) which acts as a mitogen, and antibiotic Gentamycin. The culture tubes were incubated for about 72 hours in a CO2 incubator, followed by harvesting of culture, preparation of slides, and staining using Giemsa stain.^{18,19} Karyotyping was done with the help of an automated karyotyping system (cytovision software version 7). 30 metaphase spreads were analyzed in each case for chromosomal anomalies. In suspected mosaicism, 50-100 metaphase spreads were analyzed.

RESULTS

A total of twenty-two patients with ambiguous genitalia were studied. The study group included mainly neonates, children, and two teenagers with ages ranging from 17 days to 17 years, the mean age being 2.75±19.75 months as per median and semi-interquartile range. Demographic data of the studied patients did not show any positive consanguinity or relevant maternal history.

Reports of routine serum electrolyte and hormonal studies of relevant cases revealed one case of hyperkalemia and hyponatremia, 5 cases of elevated serum 17 alpha- hydroxyprogesterone, and one case of normal 17 alpha- hydroxyprogesterone with elevated dehydroepiandrosterone (DHEA) levels associated with low concentrations of androstenedione and testosterone.

Of the 22 cases that presented with ambiguous genitalia, 12 cases (54.54%) were confirmed as genetic females with 46, XX karyotype, and 9 cases (40.9%) were genetic males with 46, XY karyotype. One neonate (4.5%) presented with karyotype 49, XXXXY (Figure 1, 2). Structural aberrations of the autosomes or sex chromosomes were not observed.

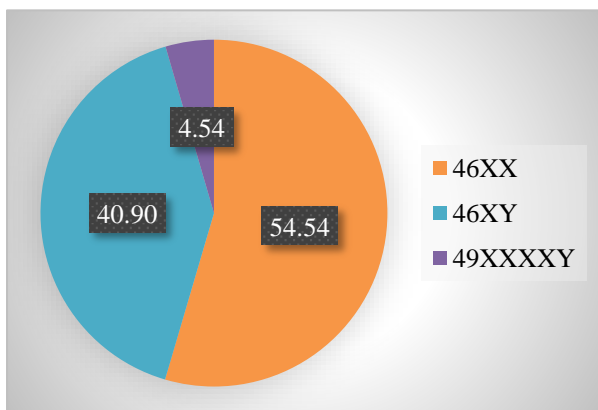


Figure 1: Karyotype percentage of patients with ambiguous genitalia.

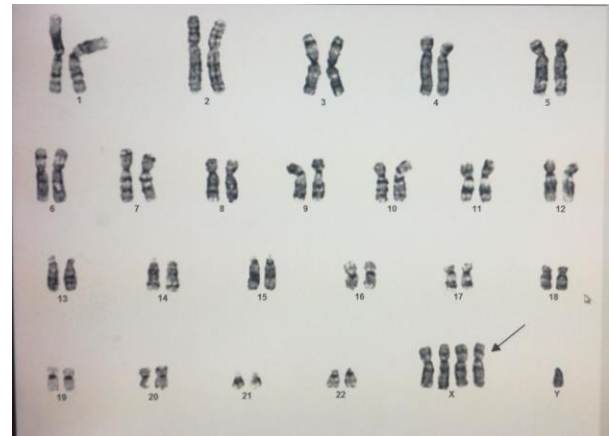


Figure 2: Karyotype showing the presence of four X and one Y chromosomes.

Genetic females presented with an enlarged phallus, urogenital sinus, fused labia, and labioscrotal folds with no palpable gonads. Two of them aged 3 and 7 years, who were sisters presented with abnormal pubic hair growth (tanner stage P3), with a score of 2 and 3 on the prader system. (Figure 3). Reports of imaging studies revealed the presence of internal genitalia in all genetic females except in one case of age 16 years who presented with primary amenorrhea.

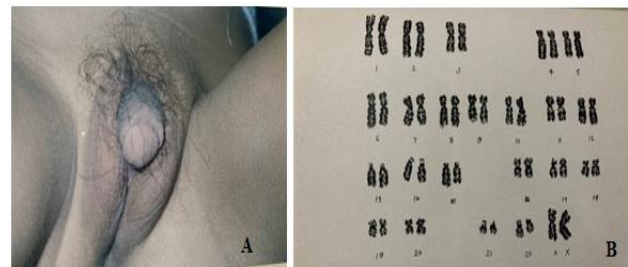


Figure 3: (A) External genitalia showing ambiguity and precocious puberty in a 3-year-old genetic female (sex of rearing-male) with CAH, due to 21-hydroxylase deficiency and (B) Karyotype of the patient showing 46, XX.

Congenital adrenal hyperplasia (CAH) was detected in 5 patients with 46, XX karyotype (41.7% of the genetic females), including the sibs with precocious puberty. Detailed clinical data of genetic females are shown in Table 1.

Genetic males presented with microphallus, penoscrotal hypospadias bifid scrotum, palpable gonads, or undescended testis. One neonate presented with phallus duplication and unilateral undescended testis. Partial androgen insensitivity syndrome (PAIS) was diagnosed in two cases (22.2% of genetic males). One case was diagnosed to have CAH due to 3 beta-hydroxysteroid dehydrogenase deficiency (Figure 4). Detailed clinical data of genetic males are shown in Table 2.

Table 1: Clinical data of 12 cases with female genetic sex.

S. no.	Age	Clinical presentation and Prader stage	Social sex	Karyotype	Diagnosis
1	Neonate	P1	UD	46, XX	-
2	Neonate	P2	UD	46, XX	CAH
3	Neonate	P3	UD	46, XX	CAH
4	Neonate	P1	UD	46, XX	-
5	1Y	P2	UD	46, XX	CAH
6	3Y	P2 with sparse hair growth	Male	46, XX	CAH
7	3.5Y	P1	Female	46, XX	Clitoromegaly
8	6Y	P1	Female	46, XX	Clitoromegaly
9	7Y	P3 with sparse hair growth	Male	46, XX	CAH
10	12Y	P2	Male	46, XX	Endodermal sinus tumour
11	16Y	P1, Primary Amenorrhea	Female	46, XX	-
12	17Y	P2, Primary Amenorrhea	Female	46, XX	-

Table 2: Clinical data of 10 cases with male genetic sex and a case of Klinefelter syndrome.

S. no.	Age	Clinical presentation	Social sex 1	Karyotype	Diagnosis
1	Neonate	Phallus, urogenital sinus	UD	46, XY	-
2	Neonate	Bifid phallus, undescended testis (left)	Male	46, XY	Phallus duplication
3	Neonate	Micro penis	UD	46, XY	-
4	2M	Micro penis, hypospadias	Female	46, XY	CAH
5	2.5M	Small phallus, hypospadias	UD	46, XY	-
6	3M	Urogenital sinus, labioscrotal folds	Female	46, XY	-
7	5M	Hypospadias	Male	46, XY	-
8	3Y	Micro penis, urogenital sinus	Female	46, XY	PAIS
9	3Y	Hypospadias, bifid scrotum	Male	46, XY	PAIS
10	Neonate	Preterm (36 weeks) Micro penis, labioscrotal folds, bilateral undescended testis	UD	49, XXXXY	Klinefelter syndrome

M–Months, Y–Years, UD – Undetected, CAH–Congenital adrenal hyperplasia, PAIS – Partial androgen insensitivity syndrome



Figure 4: (A) Ambiguous genitalia in a 2-month-old genetic male (sex of rearing-female) who was diagnosed to have CAH, due to 3 beta-hydroxysteroid dehydrogenase deficiency, (B) Karyotype of the patient showing 46, XY.

Of the 22 cases, sex for rearing was not assigned in nine cases. Eight cases of our study group were neonates, and their investigations were incomplete but routine investigations helped to diagnose two cases of CAH

among them, one presented with a salt-losing crisis. Out of the nine cases of age 17 days-1 year in which sex of rearing was not assigned, five cases were of 46, XX karyotype and included three cases of CAH, one case of isolated clitoromegaly, and one case without a definite diagnosis. Three cases with unassigned sex of rearing showed 46, XY karyotype, and no definite diagnosis was reached in them (two neonates and one infant with age two and half months).

Sex of rearing was female in seven cases, of which three were found to have 46, XY Karyotype. Of these three cases, one infant aged two months was a case of CAH due to 3 beta-hydroxysteroid dehydrogenase deficiency, one with three years of age was a case of partial androgen insensitivity syndrome (PAIS), and a three-month-old infant was without proper diagnosis. Sex of rearing was male in six cases, of which three were of 46, XX Karyotype which included 3- and 7-years old siblings with CAH and a 12-year-old case with endodermal sinus tumor.

DISCUSSION

The present study included 22 patients with ambiguous genitalia from north Kerala. The age of the patients ranged from 17 days to 17 years, and the most common age at presentation was in the neonatal period (nine cases, 40.9%), followed by infancy (five cases, 22.7%). As per cytogenetic evaluation, twelve patients were found to have normal female karyotype 46, XX (54.5%), Nine patients showed normal male karyotype 46, XY (40.9%) and one had abnormal karyotype 49, XXXXY (4.5%). This cytogenetic study revealed discrepancies in genetic sex and sex of rearing in 27% of cases and the presence of numerical anomaly of the chromosome with a rare karyotype in one case. These findings emphasize the importance of karyotyping in patients with ambiguous genitalia to confirm the genetic sex and rule out associated chromosomal anomalies.

As reported previously, the family history was relevant in our study in the case of two sisters affected by CAH.¹² A child previously affected in the family should raise the possibility of CAH, an autosomal recessive condition that causes excess adrenal androgen, which leads to genital ambiguity.²⁰ Cases of siblings affected with CAH and presented with hirsutism are already reported in the literature.¹²

46, XX karyotype was more in number in the present study (12 cases, 54.5%). Studies conducted in patients from the valley of Kashmir have reported 58% of genetic females and 42% of genetic males.¹⁶ Many of the previous authors have reported an incidence of more number of genetic females in their study group.^{5,12,16} Several authors have also reported the incidence of the mosaic karyotype.^{2,21,22} However, our study failed to detect mosaicism.

The present study of 22 cases of ambiguous genitalia detected six cases of CAH (27.3%). Yüce et al have reported 38% of CAH in the study of 21 cases.⁶ CAH is an inherited disorder due to defects in enzymes of the adrenal cortex required for cortisol biosynthesis, leading to an excess of adrenal androgens and virilization of external genitalia in the female fetus. The timing of androgen excess markedly influences the phenotypic appearance of external genitalia in newborns. In females, excess exposure to androgen in the first trimester may cause fusion of labioscrotal folds and the formation of a single urogenital sinus. Excess exposure to androgen after the critical first trimester leads to isolated clitoromegaly.^{4,20} Worldwide, the incidence of CAH is estimated at one in 15,000 but varies among communities.^{23,24} Out of the 12 cases with 46, XX karyotype in the present study, five cases were diagnosed with CAH, and the cause of CAH was a 21-hydroxylase deficiency in all these cases, based on higher levels of serum 17-hydroxyprogesterone.²⁰ Most patients presenting with ambiguous genitalia at birth are genetic females with virilizing CAH due to 21- hydroxylase

deficiency.²⁰ Two of the genetic females with CAH were neonates, and one of them presented with salt-losing. Many previous studies have reported a salt-losing variety of CAH.^{1,3}

The sex of rearing was not corresponding to genetic sex in 50% of cases of CAH. Al-Jurayyan has reported that 47.2% of cases of genetic females with CAH were wrongly assigned as males.⁴ One case with CAH in our study was a genetic male who was reared as a female, and the cause of CAH was 3 beta-hydroxysteroid dehydrogenase deficiency. Al-Mutair et al.²⁵ have reported two cases of CAH caused by 3-beta-hydroxysteroid dehydrogenase deficiency.

In this study, 22.2% of genetic males were diagnosed with partial AIS caused by partial unresponsiveness to androgen action. Previous studies also report PAIS as the most common diagnosis in genetic males. Disorders affecting the end-organ androgen receptor are referred to as the androgen insensitivity syndrome. The defect may be complete or partial, resulting in normal female external genitalia or ambiguous genitalia, respectively.^{6,25} These conditions are X-linked. Since the anti-Müllerian Hormone (AMH) action is unaffected, fallopian tubes, uterus, and upper vagina are absent.⁴ Al-Agha et al has detected 17.2% of AIS in their study and reported that 34% of the 46XY infants with no definite diagnosis could be cases with partial androgen insensitivity, a diagnosis of exclusion.⁴ Gonadal dysgenesis and gonadotropin abnormalities are also reported in genetic males.²⁶

This cytogenetic study also detected one numerical anomaly of the chromosome. A neonate presented with a micro penis, labioscrotal folds, and undescended testis. This case was a variant of Klinefelter syndrome with karyotype 49, XXXXY. Usually, the karyotype of Klinefelter syndrome is 47, XXY. 15% of Klinefelter syndrome have mosaic karyotypes. There are several variants of Klinefelter syndrome with karyotypes other than 47, XXY, including 48, XXYY, 48, XXXY, and 49, XXXXY. The additional X chromosomes, even though they are mostly inactive, cause a correspondingly more severe phenotype. Such cases show greater dysmorphism, more defective sexual development, and more severe mental impairment also. The incidence of Klinefelter syndrome is one in 2000 births (one in 1000 male births).¹¹ Incidence of Klinefelter syndrome with karyotypes 47, XXY, 46, XX/47, XXY, 45, X/47, XXY, and another numerical anomaly Edwards syndrome has been reported by previous authors.^{2,3,6,21}

In the present study, one neonate presented with a bifid phallus, a rare anomaly. It occurs once in every 5.5 million live births.²⁷ A 12-year-old genetic female with enlarged phallus and labioscrotal folds, who was reared as a male, presented with recurrent abdominal pain and was diagnosed as a case of endodermal sinus tumor. A similar case with clitoral hypertrophy has been reported in the literature.²⁸ Further, a definite diagnosis was not

reached in nine cases, which included five genetic males and four genetic females. It is reported that clinicians establish a diagnosis in only half of the cases within the neonatal period.²³ On rare occasions, the cause of ambiguity remains unexplained despite extensive studies.²⁵

The presence of an intact Y chromosome leads to maleness regardless of the number of X chromosomes. The testis determining factor is the SRY gene (Sex determining Region of Y) on the short arm of the Y chromosome.⁷ In the absence of SRY, female sexual differentiation occurs.²⁴ The 46, XX testicular sex differentiation disorder, or XX male syndrome, is a rare condition in which testicular development occurs in the absence of the Y chromosome. It occurs in one in 20,000 to 25,000 male newborns.^{6,29} Also, the 46, XX karyotype is the most common karyotype found in ovotesticular DSD.²⁰ Cytogenetic study at the chromosomal level needs more investigations to support the result. PCR analysis of the SRY gene provides information about the presence of a Y chromosome within one day although a full karyotype is required for confirmation and exclusion of mosaicism.^{6,16}

This study has detected genetic females with normal internal genitalia reared as males who require reassignment of sex. Reassignment of sex in patients with ambiguous genitalia is a very sensitive matter. Parents should be counseled, and their approval should be obtained before taking a decision.^{9,25}

Limitations

Correlation between phenotype and karyotype could not be attempted in all cases due to the unavailability of complete clinical data.

CONCLUSION

To conclude, the present cytogenetic study of 22 cases with ambiguous genitalia detected 12 genetic females, nine genetic males, and one rare variant of Klinefelter syndrome with karyotype 49, XXXXY. The most common etiology was CAH in genetic females and PAIS in genetic males. In 27.3% of cases, it was found that the genetic sex was not corresponding to the sex of rearing.

Sex of rearing should be assigned as early as possible, usually based on the internal and external genital phenotype and the results of the various investigations. In the case of a virilized female, there is fertility potential, and these babies are usually raised as girls. The decision of sex of rearing and the timing of surgery need careful consideration within a multidisciplinary environment with the full informed consent of the family. As common causes of genital ambiguity have a genetic basis, genetic counseling will be required in most cases. Also, DNA tests are important in prenatal diagnosis.

Recommendations

The authors suggest that the conventional cytogenetic method should be coupled with molecular analysis. PCR-based DNA diagnostic method using SRY gene in individuals with ambiguous genitalia plays an important role.

ACKNOWLEDGEMENTS

The authors thank the clinical team for directing the patients for karyotyping, and also the technical staff of the cytogenetic lab.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Rajendran R, Hariharan S. Profile of intersex children in south India. *Ind Pediatr.* 1995;32(6):666-71.
2. Sangeetha R, Balachandar V, Devi SM. Cytogenetic Study on Sexual Ambiguity in Humans. *Int J Hum Genet.* 2010;10(1-3):81-6.
3. Al-Agha A, Thomsett M, Batch J. The child of uncertain sex: 17 years of experience. *J Paediatr Child Health.* 2001;37(4):348-51.
4. Al-Jurayyan NAM. Ambiguous genitalia: Two decades of experience. *Ann Saudi Med.* 2011;31(3):284-8.
5. Abdulameer S. Cytogenetic Analysis of Children with Ambiguous Genitalia in Mosul. *Rafidain J Sci.* 2012;23(5):33-7.
6. Yuce H, Etem E, Ozbey U. Cytogenetic and Molecular Evaluation of Ambiguous Genitalia In Pediatric Patients. 2008;13.
7. Ellard S, Turnpenny P. Emery's Elements of Medical Genetics. 12th ed. Elsevier; 2005.
8. Kasper D, Fauci A, Hauser S, Longo D, Jameson J. Harrison's Principles of Internal Medicine. 19th ed. Macraw-Hill; 2015.
9. Ogilvy-Stuart AL. Early assessment of ambiguous genitalia. *Arch Dis Child.* 2004;89(5):401-7.
10. Kaur A, Mahajan S, Singh JR. Cytogenetic Analysis in Cases with Sex Anomalies. *Int J Hum Genet.* 2004;4(3):167-71.
11. Nussbaum R, McInnes R, Willard H. Thompson & Thompson Genetics in Medicine. 7th ed. Elsevier; 2007.
12. Abou El Ella SS, Tawfik MAM, Ellahony DM, Anees NM. Cytogenetic and Molecular study in Intersex. *Egypt J Med Hum Genet.* 2012;13(3):281-9.
13. Kumari V, A.Vani AV. Ambiguous Genitalia: A Clinical and Chromosomal study. *Int J Res Med Sci.* 2015;3(12):3743-8.

14. Hughes IA. Disorders of sex development: a new definition and classification. *Best Pract Res Clin Endocrinol Metab.* 2008;22(1):119-34.
15. Simpson JL. Disorders of the Gonads, Genital Tract, and Genitalia. In: Emery and Rimoin's Principles and Practice of Medical Genetics. Elsevier; 2013:1-45.
16. Pandith AA, Akbar S, Faheem S, et al. Molecular and Cytogenetic Evaluation of Gender in Patients Born with Ambiguous Genitalia from Different Regions of the Valley of Kashmir, North India. *J Genet Syndr Gene Ther.* 2015;06(02):6-10.
17. Abdi A, Zarbati N, Asami M, et al. Cytogenetic Study in Patients with Ambiguous Genitalia. *Sarem J Reprod Med.* 2016;1(2):79-83.
18. Arakaki DT, Sparkes RS. Microtechnique for Culturing Leukocytes from Whole Blood. *Cytogenet Genome Res.* 1963;2(2-3):57-60.
19. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res.* 1960;20(3):613-6.
20. Anhalt H, Neely EK, Hintz RL. Ambiguous Genitalia. *Pediatr Rev.* 1996;17(6):213-20.
21. Kaur A, Mahajan S, Singh JR. Cytogenetic Analysis in Cases with Sex Anomalies. *Int J Hum Genet.* 2004;4(3):167-71.
22. Abou El-Ella S, Tawfik M, Abo El-Fotoh W, Abo Howla A. Genetic evaluation of children with ambiguous genitalia. *Menoufia Med J.* 2016;29(1):79.
23. Thyen U, Lanz K, Holterhus P-M, Hiort O. Epidemiology and Initial Management of Ambiguous Genitalia at Birth in Germany. *Horm Res Paediatr.* 2006;66(4):195-203.
24. Kaye CI, Cunniff C, Firas JL, Moeschler J, Panny SR, Trotter TL. Evaluation of the Newborn With Developmental Anomalies of the External Genitalia. *Pediatrics.* 2000;106(1):138-42.
25. Al-Mutair A, Iqbal MA, Sakati N, Ashwal A. Cytogenetics and etiology of ambiguous genitalia in 120 pediatric patients. *Ann Saudi Med.* 2004;24(5):368-72.
26. Gangane SD. *Human Genetics.* 5th ed. Reed Elsevier India Pvt. Ltd; 2017.
27. Tirtayasa PMW, Prasetyo RB, Rodjani A. Diphallia with Associated Anomalies: A Case Report and Literature Review. *Case Rep Urol.* 2013;2013:1-4.
28. Abell MR, Holtz F. Ovarian neoplasms in childhood and adolescence. II. Tumors of non-germ cell origin. *Am J Obstet Gynecol.* 1965;93(6):850-66.
29. Bianco B, Christofolini DM, Ghersel FR, Gava MM, Barbosa CP. XX testicular disorder of sex differentiation: case report. *Einstein (São Paulo).* 2011;9(3):394-6.

Cite this article as: Lekha KS, Bhagyam V, Varghese PD, Manju M. Genital ambiguity: a cytogenetic evaluation of gender. *Int J Res Med Sci* 2021;9:364-70.