OPEN ACCESS

Scholars International Journal of Obstetrics and Gynecology

Abbreviated Key Title: Sch Int J Obstet Gynec
ISSN 2616-8235 (Print) |ISSN 2617-3492 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Karyotypic Analysis of Individuals with Unassigned Sex: First Report from Jammu and Kashmir, India

Wahied Khawar Balwan[1*], Neelam Saba[2]

[1]Senior Assistant Professor, Department of Zoology, Govt. Degree College Doda, Jammu & Kashmir
[2]Assistant Professor, Department of Zoology, Govt. Postgraduate College Bhaderwah, Jammu & Kashmir

DOI: 10.36348/sijog.2023.v06i12.001 | **Received:** 23.10.2023 | **Accepted:** 28.11.2023 | **Published:** 01.12.2023

***Corresponding author:** Wahied Khawar Balwan
Senior Assistant Professor, Department of Zoology, Govt. Degree College Doda, Jammu & Kashmir

Abstract

Every minute of every day, a baby is born. Most babies are easily seen to be a girl or a boy. Imagine how confusing it must be when we don't know the sex of a newborn? This is rare and it can be very upsetting for parents. During the present study, a total of 47 individuals with unassigned sex were enrolled for chromosome study. These 47 individuals in different age group were categorized into three groups namely ambiguous genitalia, Intersex and Hypospadias. In Ambiguous genitalia group, a total of seventeen individuals were enrolled for chromosome study. Of these, thirteen individuals reared as males were found to have 46, XY karyotype. In the remaining four cases reared as female sex, three were found to have 46, XX karyotype and one which was phenotypically a female was found to have 46, XY karyotype. In Intersex group, a total of fourteen individuals were enrolled for chromosome study. Of these fourteen cases of Intersex reared as male sex by their parents, thirteen were found to possess 46, XY karyotype where as one cases was found possessing 46, XX. In Hypospadias group, a total of sixteen individuals were enrolled for chromosome study and were found to have 46, XY karyotype. The purpose of the present study was to find out the chromosomal sex in these individuals. The study is first of its kind from Jammu region of Jammu and Kashmir, India.

Keywords: Unassigned Sex, Ambiguous genitalia, Intersex, Hypospadias, Karyotype, Chromosome.

Copyright © 2023 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

A baby's genetic sex is partially determined at conception when the child inherits a pair of sex chromosome namely an X chromosome from the mother and either X or Y chromosome from the father. If the baby inherits an X chromosome from both the parents, it is a genetic female (XX). If the baby inherits the Y chromosome from the father, the baby is a genetic male (XY chromosomes). Hormonal influence in-utero also plays role in determination in sex determination and reproductive development (Balwan and Saba, 2021).

During the present study, individuals with unassigned were categorized into Ambiguous genitalia, Intersex and Hypospadias.

Ambiguous Genitalia

Ambiguous genitalia is a rare birth defect in which a baby's external genitals aren't clearly male or female. The genitals may not be well formed, or the baby may have characteristics of both sexes. The outer genitalia may not match the internal sex organs. The condition, a disorder of sexual differentiation, affects an estimated 1 in 4,500 infants. Ambiguous genitalia constitute a rare phenotypic presentation of the urogenital system that can signal an underlying life-threatening disorder. Thus, it is imperative to determine the etiology as quickly as possible when ambiguity is noted. The formation of typical male or female external genitalia results from a number of genetic and physiological events starting with sex determination and progressing through differentiation of internal and external reproductive structures after a zygote is formed (Krishnan *et al*., 2019, Balwan *et al*., 2020, Balwan & Saba, 2018).

Intersex

Intersex people are born with sex characteristics (including genitals, gonads and chromosome patterns) that do not fit typical binary notions of male or female bodies. Intersex is an umbrella term used to describe a

wide range of natural bodily variations. In some cases, intersex traits are visible at birth while in others, they are not apparent until puberty. Some chromosomal intersex variations may not be physically apparent at all (*United Nations Office of the High Commissioner for Human Rights*, 2015). Intersex is a group of conditions where there is a discrepancy between the external genitals and the internal genitals (the testes and ovaries). Intersex is a complex issue, and its treatment has short and long term consequences.

Hypospadias

Hypospadias is a birth defect (congenital condition) in which the opening of the urethra is on the underside of the penis instead of at the tip. The urethra is the tube through which urine drains from your bladder and exits your body. Hypospadias is one of the most common urogenital anomalies. It is due to a failure of complete fusion of the urethral folds in the early embryonic stage (Norman, 1963). Hypospadias in the male has frequently been regarded as a mild form of intersexuality. This concept is based only upon the observation that a male with hypospadias is morphologically more similar to a female than is a male without abnormality of the external genitalia. There has been no demonstration that the presence of feminine cells causes this deviant development (Richard *et al*., 1969, Balwan & Saba, 2018). Hypospadias is common and doesn't cause difficulty in caring for your infant. Surgery usually restores the normal appearance of your child's penis. With successful treatment of hypospadias, most males can have normal urination and reproduction. Hypospadias is present at birth (congenital). As the penis develops in a male fetus, certain hormones stimulate the formation of the urethra and foreskin. Hypospadias results when a malfunction occurs in the action of these hormones, causing the urethra to develop abnormally. In most cases, the exact cause of hypospadias is unknown. Sometimes, hypospadias is genetic, but environment also may play a role.

MATERIAL AND METHOD

The study first of its kind from Jammu and Kashmir, India was carried out on patients with unassigned sex. Complete case history of patients was recorded. Blood Sample for raising lymphocyte culture of every patient was collected in heparin for chromosomal study following Moorhead *et al.*, (1960) with slight modification as done by Balwan *et al.*, (2020a, 2020b, Balwan & Saba 2018). Whole blood culture was raised separately for each patient. In each case, a total of 0.3 ml of peripheral blood was collected by venipuncture using heparinized syringe. The samples were labeled and then transferred to the culture media under very aseptic environment. Culture media was prepared by dissolving the 10ml RPMI-1640 with stable glutamine, 1ml Fetal Calf Serum (FCS), 0.2ml Antibiotic Solution containing Pencillin, Streptomycin and 0.2ml Phytohaemagglutinin-M (PHA-M) in 1000ml of autoclaved triple distilled water. After adding 0.3ml of heparinized blood in the culture medium, CO_2 was passed in these culture tubes. Then the tubes were plugged tightly and they were placed in incubator at $37^{O}C$ for 72 hours. After every 24 hours, the culture was checked for the growth of the cells and it was shaken gently for thorough mixing of cells. One hour before harvesting 0.1 ml aqueous colchicine (10µg/ml) was added. Cultures were harvested following conventional process and flame dried slides were prepared. Slides were preserved for Giemsa-Trypsin-Giemsa (GTG) banding and stained with Giemsa following Sea bright, 1971. The G-banded slides were scanned for metaphase spread under 10X magnification using Carl Zeiss AXIO Imager Metaphase analyzer. Position of well spread metaphase plates was recorded using Vernier scale on the microscope stage. The metaphase plates were analyzed in detail under 100X oil immersion objective. A minimum of 20-30 banded prometaphase and metaphase plates were captured using image analyzer through CCD camera. Well spread banded prometaphase and metaphase plates were photographed under 100X oil immersion objective and photographic prints of Metaphase spreads only were taken on 130 GSM Inkjet Photo Glossy Sheets.

Individual chromosomes were cut from the photographic prints and karyotypes were prepared according to the ideograms of the International System for Human Cytogenetic Nomenclature (ISCN, 1995) and then the karyotypes were analyzed.

RESULT AND DISCUSSION

During the present study, first of its kind, a total of 47 individuals with unassigned sex were enrolled for chromosome study. These 47 individuals in different age group were categorized into three groups:

Type	Number	Age Group
Group 1. Ambiguous Genitalia	17	(7 Days-19 Years)
Group 2. Intersex	14	(11 Months -16 Years)
Group 3. Hypospadias	16	(2 Years-19 Years)

Details of their clinical profile along with chromosome study of each group is as under:

Group 1: Ambiguous Genitalia

Ambiguous genitalia is a birth defect of the sex organs that makes it unclear whether an affected newborn is a girl or boy. In this group, a total of seven individuals having ambiguity of their genitalia were

diagnosed as cases of ambiguous genitalia. Chromosome study was carried out to find the chromosomal sex in these individuals.

Of seventeen cases, thirteen were reared as males (Fig. 1a, 1b & 1c) by their parents, whereas the remaining four individuals were reared by their parents as females (1d, 1e and 1f). However, chromosome study was felt necessary to find out the exact sex in these seven individuals.

Through chromosome study, thirteen individuals reared as males were found to have 46, XY karyotype. Thus, the chromosome study confirmed these individuals as males. In the remaining four cases reared as female sex, three were found to have 46, XX karyotype and one which was phenotypically a female found to have 46, XY karyotype.

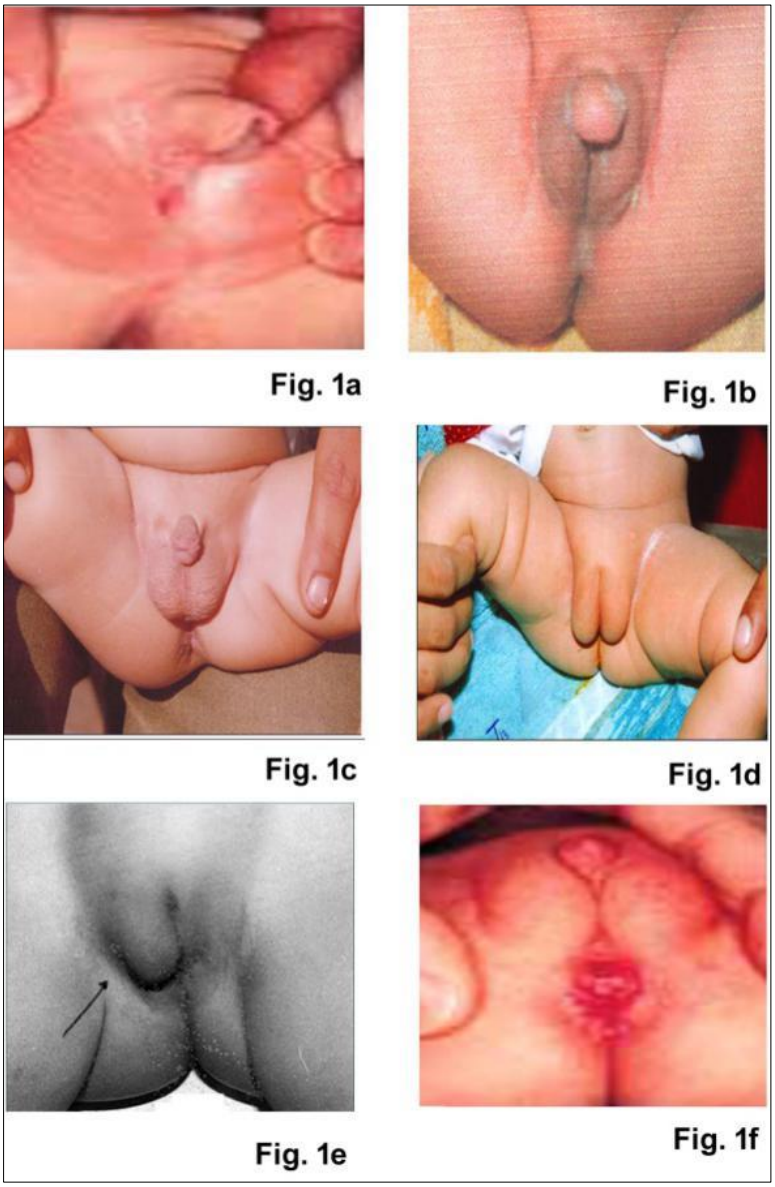

Fig.1a-1f: Phenotype of Ambiguous Genitalia

Details of the female who was found to have the karyotype of the males has been discussed:

Clinical Profile

The propositus was the 1st daughter of non-consanguineous couple from rural area. She was phenotypically a female and was hospitalized for evaluation of Ambiguous Genitalia at the age of 16 Years. Her father and mother were healthy and had no history of taking drugs during pregnancy. She had feminine appearance together with small breast development and relatively scanty axillary and pubic hairs. She had abnormally developed clitoris and vulva. According to abdominal ultrasonography, Uterus and Cervix could not visualized. A single 2.5x1.2cms structure with few small follicles was seen in right

adnexia. Right and left ovary not visualized. No testicular tissue seen in lower abdomen or inguinal region. No abnormal vascularity could be seen on colour Doppler study.

Chromosomal Profile

Some of the well spread G-banded metaphase plates were selected for study of the chromosome number and for the preparation of their karyotypes. Every well spread metaphase plate contained 46 chromosomes (Fig.2a).

Karyotypic Analysis

Some of the G-banded metaphase plates were karyotypes. Every karyotype contained 44 autosomes and a pair of heteromorphic sex chromosome-XY. All the 46 chromosomes paired normally and they had normal morphology and represent the karyotype of Normal Male i.e. 46, XY (Fig. 2b).

Abnormalities of the external genitalia could be due to the XY sex chromosome constitution.

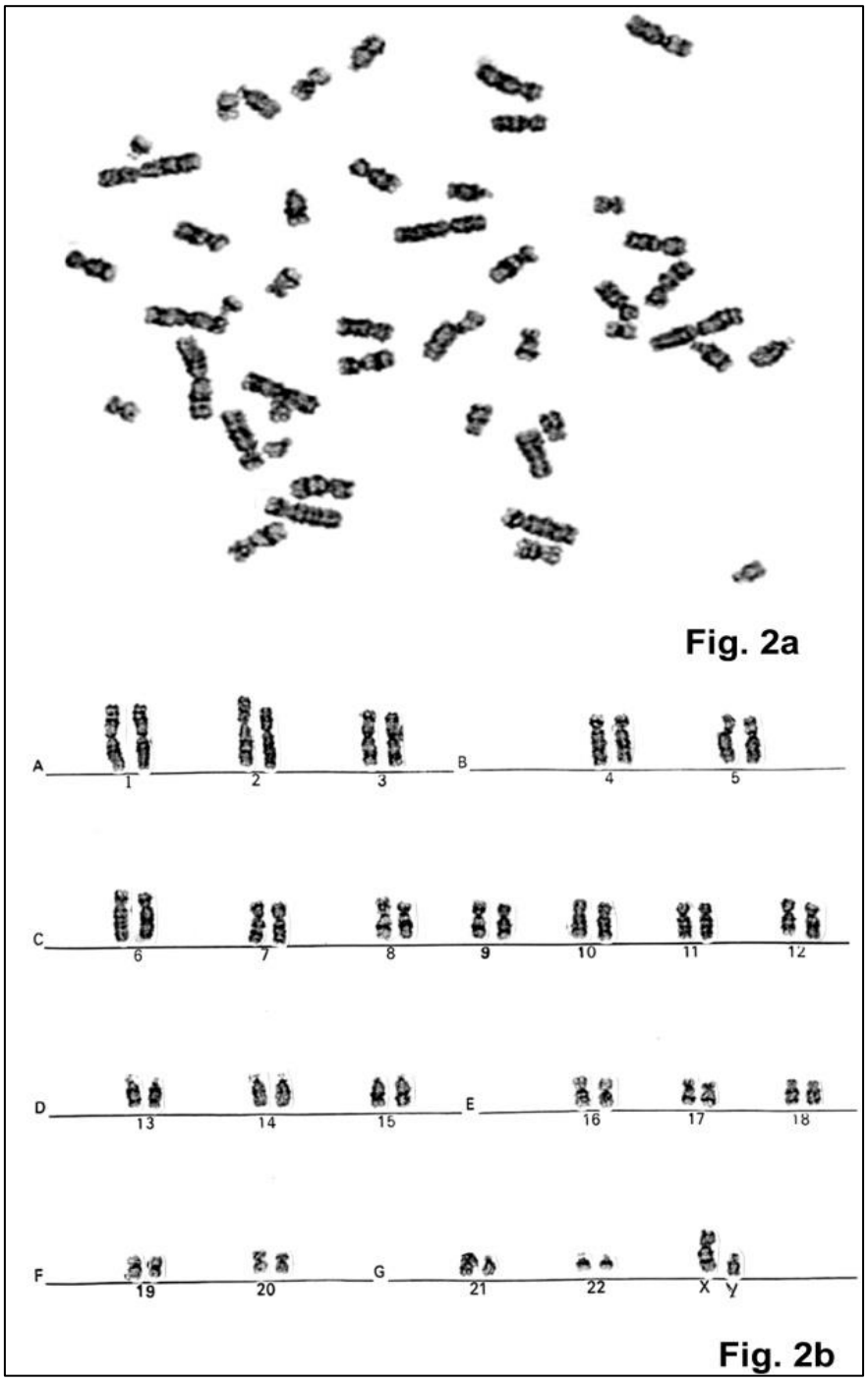

Fig. 2a & 2b: Complement and Karyotype of Phenotypically Female Patient

GROUP 2: INTERSEX

Intersexuality is the condition in which chromosomal sex is inconsistent with phenotypic sex or in which the phenotype is not classifiable as either male or female. In this group, a total of fourteen individuals clinically diagnosed as cases of intersex were enrolled for chromosome study. The objective was to find out the chromosomal sex in these individuals. These were enrolled for chromosome study as cases of Intersex to find out the chromosomal sex in these individuals. All these fourteen individuals were reared as male sex by their parents. However, chromosome study was felt necessary to find out the exact sex in these four individuals.

Of these fourteen cases of Intersex (Fig. 3a, 3b, 3c, 3d) reared as male sex by their parents, thirteen were found to possess 46, XY karyotype where as one cases was found possessing 46, XX.

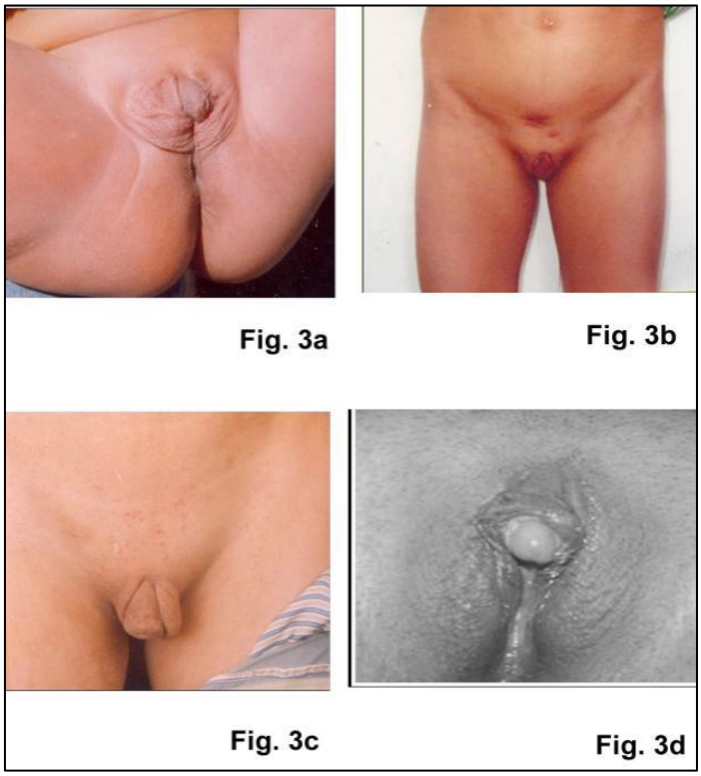

Fig. 3a-3d: Phenotype of Intersex

Details of the male who was found to have the karyotype of the females has been discussed:

Clinical Profile

The proposita (Fig. 3d) was the 2nd son of non-consanguineous couple from urban area. He was hospitalized for evaluation of Intersex condition at the age of 16 Years. His father and mother were healthy and had no history of taking drugs during pregnancy. She had a masculine appearance together with breast development and relatively no axillary and pubic hairs. He showed clitoral megaly. According to USG report testes invisible, both ovaries seen, left ovary 16.7 x 18.3 mm and right ovary 10.3 x 30.4 mm in size and presence of broad vaginal pouch, ureters not dilated, urinary bladder is normal, well placed regular, small prostratic tissue seen. Menses had also started.

Chromosomal Profile

Some of the well spread GTG banded metaphase plates were selected for study of the chromosome number and preparation of their karyotypes. Every well spread metaphase plate contained 46 chromosomes (Fig.4a).

Karyotypic Analysis

All the cells studied were karyotyped. Every karyotype contained 44 autosomes and a pair of homomorphic sex chromosome XX. All the 46 chromosomes paired normally and they had normal morphology and represent the karyotype of Normal Female i.e. 46, XX (Fig.4b).

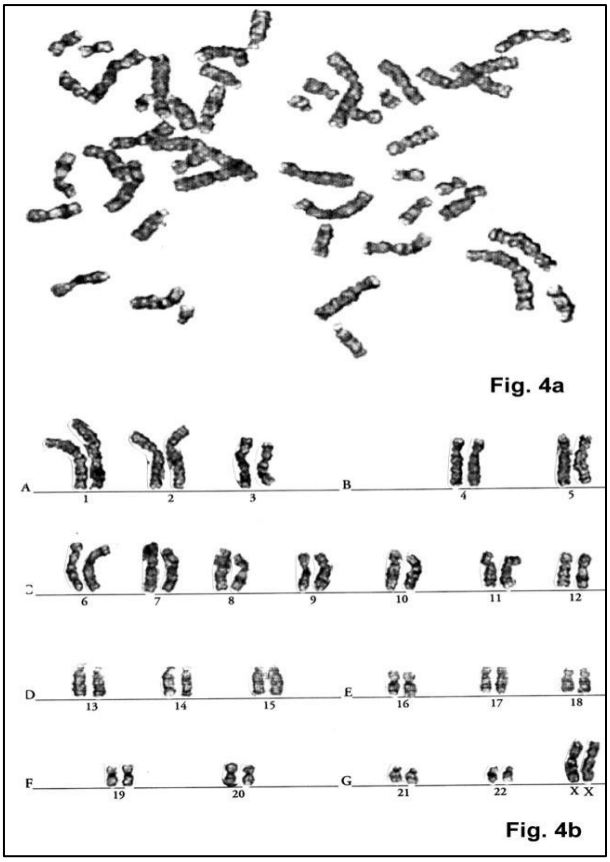

Fig. 4a-4b: Complement and Karyotype of Phenotypically Male Patient

Group 3: HYPOSPADIAS

Hypospadias is a birth defect of the urethra in the male that involves an abnormally placed urinary meatus (opening). In this group, a total of sixteen individuals (Fig.5) with Hypospadias (Fig. 5a, 5b, 5c, 5d, 5e and 5f) were enrolled for chromosome study to find out the chromosomal sex in these individuals. All the Sixteen individuals were reared as males by their parents. However, chromosome study was felt necessary to find out the exact sex in these sixteen individuals. All the sixteen individuals were found possessing 46, XY karyotype (Fig. 6a & 6b).

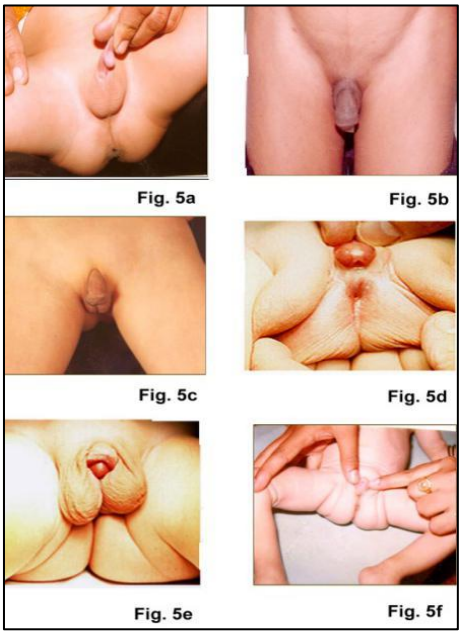

Fig. 5a-5f: Phenotype of Hypospadias

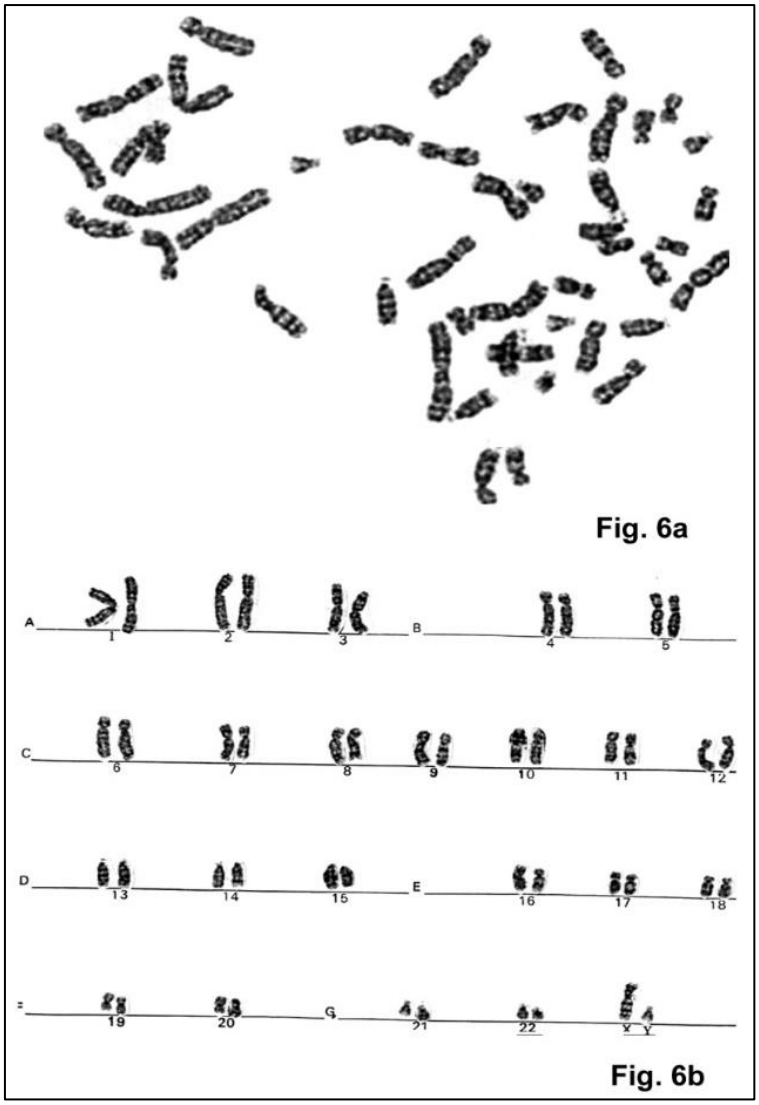

Fig. 6a-6b: Complement and Karyotype of Hypospadias Patient

CONCLUSION

Individuals with unassigned sex can be very upsetting for parents. As a conclusion, our study focused on to find out the chromosomal sex in individuals with unassigned sex, so that can go for further treatment with the help of doctors for correction of sex. Moreover, when individuals with unassigned sex approach the doctor, doctors should not hesitate to inform the parents about their child's condition and proceed to identify and diagnose the cause first then the gender. Thus, chromosome studies are always helpful to find out the exact sex of an individual.

Conflict Of Interest: Authors have no conflict of interest.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Zoology, University of Jammu, Institute of Human Genetics, University of Jammu and Govt. Medical College Jammu for providing the facilities in carrying out the present study. Authors are also thankful to Dr. Subhash Gupta, Ex-Professor, University of Jammu for his support wherever required.

REFERENCES

- Balwan, W. K., & Saba, N. (2018). Chromosome study in suspected cases of Turner's syndrome from Jammu region of Jammu & Kashmir. *Journal of Advanced Zoology , 39*(1), 32-36.
- Balwan, W. K., & Saba, N. (2021). Study of clinical aspect, genetic aspect, genetic counselling and prevention of Down's syndrome. *Indian Journal of Adult Education*, 82(1A).
- Balwan, W. K., Balwan, W. K., Saba, N., & Jamwal, K. S. (2020). Cytogenetic investigation of cases with primary amenorrhea in Jammu Region of Jammu and Kashmir. *Int J Zool Res*, *10*(2), 1-8.
- Balwan, W. K., Saba, N., & Gupta, S. (2020b). Chromosome study in individuals with unassigned sex from Jammu region of Jammu and Kashmir, India. *Wesleyan Journal of Research*, *13*(36), 52-61.

© 2023 | Published by Scholars Middle East Publishers, Dubai, United Arab Emirates

- Fane, L. R. (1969). Chromosomes and sex in Hypospadias. *Pediatrics*, *44*(5), 774-775.
- Juberg, R. C., Jewson, D. V., Taylor, M. B., & Moore, V. L. (1969). Chromosome studies in patients with hypospadias. *Pediatrics*, *43*(4), 578-582.
- Krishnan, S., Meyer, J., & Khattab, A. (2019). *Ambiguous genitalia in the New born. In*: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MD Text.com, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279168/.
- Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. T., & Hungerford, D. A. (1960). Chromosome preparations of leukocytes cultured from human peripheral blood. *Experimental cell research*, *20*(3), 613-616.
- Norman, A. P. (1963). *Congenital Anomalies in Infancy, Ch. 6, Davis*, Philadelphia.
- Seabright, M. (1971). A rapid banding technique for human chromosomes. *The Lancet*, *298*(7731), 971-972.
- *United Nations Office of the High Commissioner for Human Rights. 2015. Free & Equal Campaign Fact Sheet: Intersex.*