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The Chromosomal Abnormality Diagnosis Case Study and Other Essential Profile Study, A Review of Ambiguous or Atypical Genitalia Child Patients Which is Important to Help Them in Future

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***Abstract*— Disorders of Sex Differentiation or Development, and Ambiguous Genitalia arise when a child's gender is unclear at birth and the genitalia may not clearly show as male or female which is known as Atypical Genitalia. When a genetic female fetus is exposed to male hormones while a genetic male fetus lacks male hormones. 46, XY unvirilized male syndrome and 46, XX virilized female syndrome. This Syndrome Is Related to Indeterminate Genitalia. a field of genetics that studies the structure and function of cells and chromosomes by microscopic analysis of G band banding patterns. Cytogenetics is also a branch of genetics dealing with the research (microscopic inspection of G banding patterns) of the structure and function of cells and chromosomes. Diseases brought on by omission or inclusion. Parts of cytogenetics or whole chromosomes by the diagnosis of postnatal, prenatal, or gene mapping, or altering the centromere's position, are important in this condition. Diseases brought on by the loss or insertion of significant chromosomal segments or entire chromosomes can be diagnosed by postnatal, prenatal, gene mapping, or by moving the centromere. Investigating Chromosomal Abnormalities and Additional Crucial Profiles in Patients with Ambiguous or Atypical Genitalia. Karyotype preparation involves creating slides and comparing them to the case studies. In the current investigation, two individuals with ambiguous genitalia and a female phenotypic have been noted.**

***Keywords*— Cytogenetics, Chromosomal Abnormality, Disorder of the Sexual Differentiation or Determination (DSD), Ambiguous or Atypical Genitalia, 46, XX Virilized female and 46, XY unvirilized male Syndromes, Karyotyping, G Banding.**

I. INTRODUCTION

Ambiguous or atypical genitalia is a condition that arises when a child's gender is unclear at birth and their genitalia may not clearly show as male or female. Ambiguous Genitalia then develops together with Disorders of Sex Development [1]. When a genetic female fetus is exposed to male hormones while a genetic male fetus lacks male hormones. For parents, the ambiguous genitalia can be a traumatic event. Ambiguous genitalia are a surgical specialist of pediatrics urology that treats genitourinary system abnormalities in children [2]. Boys and girls from infancy to the early stages of adulthood can receive treatment from pediatrics urologists. The majority of frequent issues are related to urinary abnormalities. The Disorder of Sexual Differentiation and Embryology (DSD), commonly referred to as the Disorder of Sexual Determination, is a category of disorders that fall into four main categories: 46, XY unvirilized man; 46, XX virilized female. Syndromes and the Conditions Associated to Ambiguous Genitalia [1]. Genetic chromosomal disorders and gonadal differentiation. Determination of chromosomal sex at conception, gonadal differentiation at 6-7 weeks of gestation, and phenotypic sex determination at 8–12 weeks of gestation are the three steps of sexual differentiation [2]. Cytogenetics is also a branch of genetics dealing with the research (microscopic inspection of G banding patterns) of the structure and function of cells and chromosomes. Diseases brought on by omission or inclusion. Parts of cytogenetics or whole chromosomes by the diagnosis of postnatal, prenatal, or gene mapping, or altering the centromere's position, are important in this condition [3]. The frequency of DSD appearing at birth is estimated to be 0.018% (1.8 per 10,000 live births) in the prevalence rate of ambiguous genitalia. In less than half of the cases, a conclusive diagnosis is reached [5]. 1) Early in fetal development, the tissue that will become the gonads (ovaries or testes) has undifferentiated potentiality, depending on the genetics of the fetus. This is one of the causes of ambiguous genitalia. mutations in certain genes linked to ambiguous genitalia, DSD-46, XX virilized female, 46 unvirilized male insensitivity disorders [3]. The adrenal glands produce too many male hormones (androgens) as a result of non-genetic circumstances causing Ambiguous Genitalia, a disorder where Congenital Adrenal Hyperplasia has particular manifestations. The most typical reason for congenital ambiguity at birth Ohas's deficiency (SERUM 21) is the most prevalent autosomal recessive type exposure of unborn children to male hormones from some medications that either stimulate or contain male hormones [4]. A pregnant woman's growing female genitals may become more masculine as a result of her body producing more male hormones. A growing infant may potentially be exposed to excess male hormones if the mother suffers from a hormone-producing ailment [4]. The female ambiguous genitalia, in which the clitoris enlarges and

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resembles a little penis [5]. A urethral aperture may occur below, above, or along the clitoris' surface. Labia could appear to have a scrotum. A male infant with non-descending testicles may be assumed. One may feel a lump of tissue in the labia, which gives the appearance of a testicles-filled scrotum [6]. Male ambiguous genitalia are characterized by a tiny penile tissue that resembles an enlarged clitoris. The opening of the urethra can occur below, above, or along the penis. can be as low as the peritoneum, giving the impression that the baby is female. Looks like labia, perhaps a little divided scrotum. It is not unusual to find non-descending testicles [6].

II. OBJECTIVE

To Study the Chromosomal Abnormality and Other Essential Profile in Patients with Ambiguous Genitalia, I have to do Karyotyping of normal peripheral blood and some patient's peripheral blood and compairing with those normal blood chromosome with the patients' DSD blood chromosome.

III. MATERIALS AND METHODS FOR MY STUDY:

a) Materials needed to prepare a blood sample: 1.) Blood samples from patients and normal people, 2.) Sanitized Needle 3.) A tourniquet 4.) 70% alcohol, and 5.) sodium heparin, an anticoagulant.

b) Material needed for Culture Media: 1) RPMI powder, 2) A glass pipette, 3) a magnetic stirrer, 4) one liter of deionized water,5) ten milliliters of heparin,6) one milliliter of penicillin, 7) one milliliter of streptomycin, and so on.

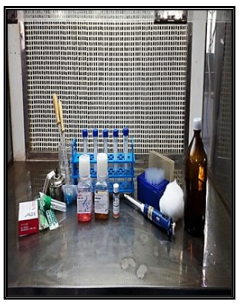
c)Supplies needed for harvesting slide preparation, inoculation, and initiation: 1.) Centrifuge, 2.) Incubator 3.) Colcimid Soln., 4.) Hypotonic KCl solution, 5.) Adhesive 6.) glass slide; 7.) Pasteur pipette; and 8.) Giemsa stain, or stain indicator. 9. Wash buffer, or phosphate buffer 10.) Trypsin.

d)Preparation of the Karyotype: Getting a Blood Sample Ready: - Actions: I) The patient's arm is punctured in vain to extract 2 milliliters of blood. II) Anticoagulants are then combined with the blood. Setting Up the Cultural Media: - Actions: 1.) In a glass pitcher with a magnetic stirrer, combine RPMI powder, one liter of deionized water, ten milliliters of heparin, one milliliter of penicillin, and one milliliter of streptomycin. Setting Up the Working Media: - PHA and NaHCO3 are combined with the stock medium specified above. 2.) Millipore filters were used. 3.) Dispersed and kept in sterile bottles.

E) At the Starting Point: 1.) Mark the patient's name and the specimen number on the container that holds the culture medium. 2.) Six milliliters of the working medium are poured into the tube in a laminar airflow. 3) Inoculation: 0.8 ml of patient blood is inoculated dropwise into the tube holding the working medium and allowed to incubate for 69 hours.

F) Harvesting: - Procedure: - Prior to beginning, we must apply a fixative prepared in a 3:1 ratio for fixation. A.) Colcimid Addition- This inhibits cell development during metaphase, also known as mitotic arrest. B.) Hypotonic Treatment: The hypotonic solution is pre-wormed to 37 degrees Celsius. C.) Centrifuge the sample for 10 minutes at 1000 rpm. D.) Pipetted off is the supernatant. Next, 5-7 milliliters of KCl are added. [Three times] E.) The incubation temperature was 37 °C. Five to six drops of newly made fixative. F.) Centrifuge it once again for 10 minutes at 1000 rpm. F.) The tubes have the opportunity to stand at room temperature for ten minutes. Chilled the tubes in the refrigerator.

G) Preparing Slides: 1. Take out the centrifuge tube from the refrigerator and use it for removing the serum from the isolated blood. 2.) We're required to perform a fixative wash before beginning this experiment. 3.) Remove the supernatant by lowering the spin. 4.) Refill the pellet with multiple times as much of the freshly prepared fixative. 5.) Gently blend the mixture of suspension and pellet together. Then set it down on the cold slide. 6.) Place it on a hot plate heated to 45 degrees Celsius for the entire day. Fixative: (30=methanol/10=acetic acid). **The Giemsa Stain**: This substance, or a mixture of glycerin, methanol, methylene blue, and eosin, is used to colorize chromosomes and tissues that form blood cells in order to facilitate microscopic investigation and identification. A gram of finely ground Giemsa powder is included in the standard Giemsa stain. 60 milliliters of well-mixed glycerol and 50 milliliters of well-mixed methanol were utilized over a period of {7 days}. Use 10 or 15 milliliters of stock and 40 or 35 milliliters of buffer {for two days} when using Giemsa stain. before being placed in a coupling jar, it is instantly prepared and filtered. 2.) You must keep Wash Buffer and Trypsin in different jars. Trypsin must first be used to clean the slide. 3.) Allow the Slide to sit in the Giemsa Stain for twenty minutes. After that, wash it with water. Then, set it aside to dry on the hot plate. For seven days, combine 50 ml of well-mixed methanol and 60 ml of well-mixed glycerol. Ten or fifteen milliliters of stock should be mixed with forty or thirty milliliters of buffer {for two days} when using Giemsa stain. It should be filtered and prepared shortly before being transferred to a Coupling Jar. 2.) Trypsin and Wash Buffer must be kept separately in jars. Trypsin and wash buffer must first be used to clean the slide. 3.) Leave the Slide in the Giemsa Stain for a full twenty minutes. After that, wash it with water. Next, set it on the heated surface plate to air dry.



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Figures 1: (Picture 1-15) These figures can explain the whole processes is going to do Karyotyping where prepare a blood sample, culture media, harvesting slide preparation, initiation, inoculation, staining and see under the microscope.

IV. LITERATURE REVIEW

In the journal "Prevalence rate of genitalia malformation in Iranian children: findings of a nationwide screening survey at school entry. " By Amir-Mohammad Armenian, Roya Kelishadi1, Gelayol Ardalan2, Mahnaz Taslimi3, Majzoubeh Taheri2, Mohammad-Esmaeil Motlagh4Department of Neonatology, 1Professor of Pediatrics, Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, 2Bureau of Population, Family, and School Health, Ministry of Health and Medical Education, Tehran, 3Department of School Health, Bureau of Health and Fitness, Ministry of Education, Tehran, 4Department of Paediatrics, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran and Bureau of Population, Family, and School Health, Ministry of Health and Medical Education, Tehran, Iran. Received: 22.03.2013, Accepted: 25.08.2013. We can see its written that One of the most momentous occasions in a family's history is the birth of a child, and the first thing people usually wonder is, "Is it a boy or a girl?" Medical professionals and parents are frequently taken aback when they discover a newborn with unclear external genitalia. Most newborns have a clear sex designation at birth. On the other journal like "Accuracy of Ultrasonic Detection of The Uterus in Normal Newborn Infants: Implications for Infants with Ambiguous Genitalia". Received: 16/08/07; Accepted: 24/10/07 has written in the Outcome Approximately one in 5000 newborns are born with ambiguous genitalia each year; if at all feasible, sex assignment must be made right away. We looked at whether transabdominal ultrasonography using a high-resolution linear array transducer operating at 7.5 MHz would improve the newborn's uterine detection accuracy. 100 moms who delivered normal, term babies (> 36 weeks and > 2500 g) provided informed permission for the study. Every newborn was positioned in an unlabeled bassinet, their external genitalia were concealed, and an ultrasound examination was conducted by an examiner (R.S.) who was not aware of the infant's gender. Also, we can see in the journal by Ostrow V, De Luca F. "Long term follow-up of a child with ambiguous genitalia, mixed gonadal dysgenesis, and unusual mosaicism." Case Reports J Pediatr Endocrinol Metab. 2009 Sep;22(9):863-6. doi: 10.1515/jpem.2009.22.9.863. PMID: 19960897. An irregular and asymmetrical gonadal development is known as mixed gonadal dysgenesis (MGD). Although 45,X/46,XY mosaicism is usually linked to this illness, reports of other karyotypes are few. MGD is characterized by a very varied phenotype, albeit ambiguous genitalia are typically present. Furthermore, stigmata associated with Turner's syndrome are seen in many patients with MGD. We report on an MGD patient whose karyotype was 45, X/47, XYY, with most of the cell lines being 47, XYY. The long-term follow-up of a patient with ambiguous genitalia who was diagnosed at birth with 45, X/47, XYY mosaicism is described in our study for the first time, as far as we know. In the journal of case report by Mazen I, Hafez M, Mamdouh M, Sultan C, Lumbroso S. "A novel mutation of the 5alpha-reductase type 2 gene in two unrelated Egyptian children with ambiguous genitalia." J Pediatr Endocrinol Metab. 2003 Feb;16(2):219-24. doi: 10.1515/jpem.2003.16.2.219. PMID: 12713261. We can see in this journal to make an etiological diagnosis in two unrelated Egyptian children with ambiguous genitalia using biochemical and molecular investigations. Two XY patients were referred: one, who was 14 years old, had delayed menarche and puberty, while the other, who was 4 months old, had unclear genitalia. The plasma levels of dihydrotestosterone (DHT) and testosterone (T) were measured at resting and after HCG stimulation. The five exons of the 5alphaR type 2 gene and exons 2 through 8 of the androgen receptor gene were sequenced directly. The high T/DHT value in the first case showed 5alphaR deficiency, but the lack of paternal

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consanguinity and normal T/DHT value in the second patient suggested androgen insensitivity. A homozygous A --> G mutation in exon 3 that substituted an aspartic acid for the asparagine residue at position 160 was found in both individuals. Both patients' parents carried the N160D substitution heterozygous. 1) We provide a novel mutation that broadens the range of genetic abnormalities associated with 5alphaR deficiency. 2) Although the two individuals were referred at quite different ages, their clinical presentations suggest phenotypic diversity for the same mutation. 3) These findings highlight how challenging it is to diagnose 5alphaR deficiency using just clinical and biochemical criteria. The only conclusive method for diagnosing ambiguous genitalia is still molecular analysis.

V. RESULTS

Observing these slides in 1.) A microscope is used to examine the slides. The chromosome ought to become a deep shade of purple. 2.) Next, chromosomal counting and analysis are completed. 3.) Photographs of the metaphases show the possibility of a Karyotype.

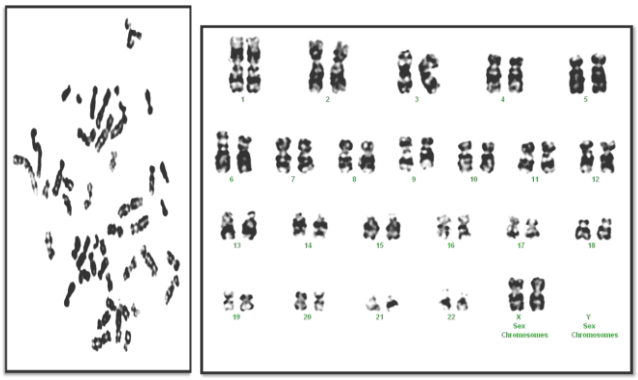

Figure 2: Normal Female Blood Sample Metaphase spread and Karyotype where 46 xx chromosomes can be seen (https://www.ncbi.nlm.nih.gov/books/NBK22266/)

- Difference with two Case Studies:

○ Case Study 1: Details of Patient

Name: **********

Age/Sex: 4years 1months/Female

Indication: (?) Ambiguous genitalia

Process: 72 hrs. stimulated culture-GTG Banding

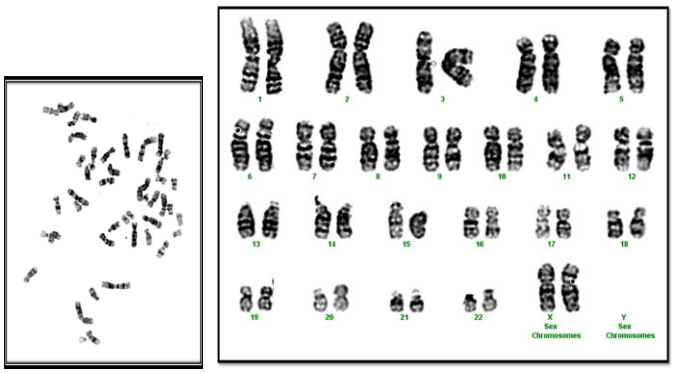

Figure 3: Peripheral Blood Sample Karyotype 46, XX virilized female. Syndromes and the Conditions Associated to Ambiguous Genitalia . Interpretation: (https://www.ncbi.nlm.nih.gov/books/NBK22266/)

In the Interpretation of this Chromosome research identified total 46 chromosomes, consisting of 44 autosomes and two sex chromosomes. With reference to the patient's history, FISH (Fluorescent in-situ hybridization) or CGH (Comparative Genomic Hybridization) should be administered to rule out submicroscopic chromosomal rearrangement. Recommended is a clinical connection.

○ Case Study 2: Details of Patient

Name: **********

Age / Sex: 2 Years 7 Months / Female

Indication: (?) Ambiguous genitalia

Process: 72 hrs. stimulated culture-GTG Banding

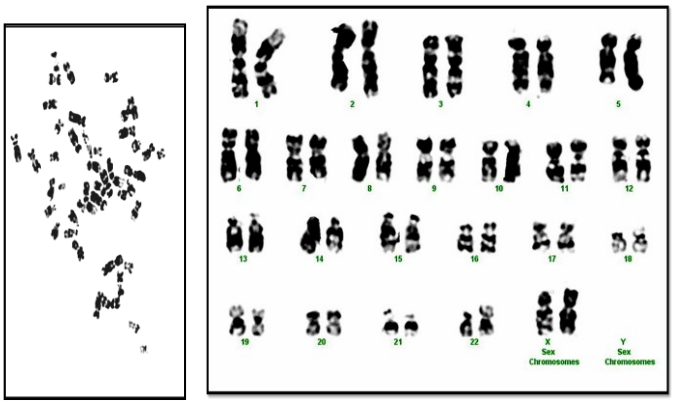

Figure 4: Peripheral Blood Sample Karyotype 46, XX virilized female. Syndromes and the Conditions Associated to Ambiguous Genitalia. Interpretation: (https://www.ncbi.nlm.nih.gov/books/NBK22266/)

In the Interpretation of this Chromosome assessment discovered a total of 46 chromosomes, including 44 autosomes and two sex chromosomes. Her clinical results (phenotype) suggest that she may be either (?) transgender or (?) intersex. DNA analysis was recommended to discover Y-chromosome translocation, which might aid with future hormonal treatment. Recommended is a clinical connection.

VI. DISSCUSION

Except in rare cases, the presence or absence of a "Y" chromosome determines an individual's phenotypic sex. Two instances with ambiguous genitalia and a female phenotype were seen in the current investigation. A chromosomal examination showed normal chromosomal components; however, it is necessary to rule out any abnormalities based on the patient's medical history. Additional sub microscopic chromosomal rearrangement aided by sophisticated, contemporary cytogenetic techniques. at my view, the following therapies—psychological counselling, hormonal therapy, and genetic counselling for family members—should be administered at the designated location. Because of the potential social and psychological repercussions of this condition, parents should make a decision about whether to raise the kid as a boy or girl as early as possible. In the first several days of life, if possible. This is a significant choice; therefore, parents should take their time.

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VII. CONCLUSION

In my work done in the Lab of Department of Genetics (2015) in Institute of Genetic Engineering I have found the interesting concluded area that should be discussed. The current study highlights once more how important cytogenetic studies are for individuals who have unclear genitalia. Other hormonal profiles and confirmatory banding methods are quite important for subsequent treatment. The early contact to a medical professional where the concerned about the appearance of the child's external genitalia, or the baby has to be taken more than two weeks to go back to the weight at birth. The person is throwing up. It appears parched. reduced appetite, blue moods, and difficulty breathing. ----It is possible to find ambiguous genitalia during the initial well-baby examination. Thus, serum electrolytes, blood sugar, hormonal research, karyotyping (G-banding), ultrasonography, Genitogaphy, sinogram, CT, MRI, exploratory laparotomy/ laparotomy, and other methods should be used for the diagnosis test.

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