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Disorder of Sex Development and Associated Anomalies: A Cytogenetic study

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ABSTRACT

The correct spatiotemporal order and coordination of mutually antagonistic activating and repressing elements are essential for normal sex development. These elements control the unipotent gonad's commitment to the binary pathways that control typical sex development. Usually, the Y chromosome's SRY gene sets off a series of biochemical processes that result in the development of male sex. The term "disorders of sex development" refers to a broad category of congenital illnesses that are linked to abnormal internal and external genital development.

Generally speaking, these illnesses are ascribed to variations from the normal course of sex development. Chromosome, gonadal, and anatomic anomalies are some of the categories into which disorders of sex development can be divided. Novel genetic variations among DSD patients have been found using genomic methods like next-generation sequencing techniques and microarray investigations. Above all, patient care must be tailored to each patient, particularly when it comes to choices on sex of raising, surgery, hormone therapy, and the possibility of preserving fertility.

Therefore, the present study was undertaken to study the disorder of sex development and associated anomalies.

1. INTRODUCTION

Disorders of sexual development (DSD) refer to a group of congenital conditions characterized by atypical development of internal and external genital structures conditions which may result from genetic variations, hormonal influences, or disruptions in developmental programming where individuals are identified at birth or later in life due to ambiguous external genitalia and may also affect the fertility of an individual [1].

There data on the incidence of DSDs remains limited where overall incidence ranges from 1 in 4500 [2] to 1 in 5,500 [3,4]. Congenital adrenal hyperplasia (CAH) and mixed gonadal dysgenesis are the most common causes of DSDs, constituting approximately over 50% of all cases of genital ambiguity in the newborn period [5]. The incidence of CAH and mixed gonadal dysgenesis worldwide is 1:15,000 and 1:10,000, respectively, but varies considerably among different populations [6,7].

Ambiguous genitalia is not a disease in itself but rather a sign of an underlying condition affecting sexual development, known as a disorder of sexual development. A thorough understanding of sex determination and differentiation is crucial to guide appropriate investigations and accurately establish a diagnosis.

An individual's genetic sex, established at fertilization, initiates a cascade of developmental events. This initial determination dictates the fate of the gonads, subsequently guiding the formation of internal and external genitalia. Consequently, phenotypic sex becomes apparent later in gestation, beginning around 5-6 weeks.

The urogenital ridge, derived from the mesonephros, serves as the origin for both male and female gonadal development. Around four weeks post-fertilization, primordial germ cells migrate from the yolk sac to this ridge. The ridge also contains precursors for steroid-producing cells (theca/Leydig) and supporting cells (follicular/Sertoli).

The undifferentiated gonads formed on these ridges have the potential to develop into either ovaries or testes. While ovarian development was once considered a default pathway, current research indicates a more active process, though the specific ovarian-determining genes remain under investigation. In contrast, testicular development is an active process that requires the expression of the primary testis-determining gene, SRY, along with other testis-forming genes such as SOX9. Additionally, the formation of the testis and male internal and external genitalia depends on the activity of male pathway transcription factors, including SF1 and WT1.

Crucially, the mesonephric origin of both gonads and fetal adrenals leads to shared developmental pathways. Consequently, genes like SF1 and DAX1 are expressed in both tissues, and mutations in these genes can disrupt both adrenal and gonadal development. Similarly, the shared expression of genes like WT1 in the kidneys and gonads explains the association between renal conditions, such as Wilms' tumor, and gonadal dysgenesis, as seen in Denys-Drash syndrome.[8]

DAX1 and Wnt4 act as potential inhibitors of testis development. Overexpression of these genes in 46XY males has been associated with impaired gonadal development and reduced virilization, leading to gonadal dysgenesis and, in some cases, complete sex reversal.

Disruptions in genes involved in gonadal differentiation, whether due to mutations or duplications, can lead to gonadal dysgenesis and abnormalities in the development of internal and external genitalia.

Wnt4 also plays a crucial role in the development of the female reproductive tract. In the absence of anti-Müllerian hormone (AMH/MIS) and testosterone, the Müllerian ducts differentiate into female structures, while the Wolffian ducts regress.[9]

During fetal development, Sertoli cells within the testes secrete anti-Müllerian hormone (AMH), leading to the regression of Müllerian ducts, which would otherwise form female reproductive structures. Therefore, in a newborn with ambiguous genitalia, the absence of a uterus strongly suggests the presence of functional testicular tissue and, specifically, functional Sertoli cells.

The Wolffian ducts, under the influence of testosterone from Leydig cells, differentiate into the internal male genitalia. Testosterone is subsequently converted to dihydrotestosterone (DHT) by 5α-reductase, a crucial step for the masculinization of the external genitalia, beginning around the sixth week of gestation.

The degree of masculinization is determined by both fetal androgen levels and tissue sensitivity to these hormones. Consequently, any disruption in this pathway can lead to ambiguous genitalia. These disruptions can arise from various factors, including:

- Genetic mutations
- Chromosomal abnormalities (e.g., 46XY/46XX or 45X/46XY mosaicism)
- Hormonal imbalances
- Androgen insensitivity syndromes (end-organ unresponsiveness)

These disruptions can manifest as under-virilization in 46XY individuals, virilization in 46XX individuals, or, rarely, true hermaphroditism. True hermaphrodites possess both ovarian and testicular tissues, which may be found in separate gonads or combined as ovotestes.[10]

Ambiguous genitalia in genetic females (XX chromosomes) can arise from:

- Congenital Adrenal Hyperplasia (CAH): Certain forms of CAH lead to overproduction of androgens (male hormones) by the adrenal glands.
- Prenatal Androgen Exposure: Exposure to male hormones or androgen-like drugs during fetal development can masculinize female genitalia.
- Maternal Androgen-Producing Tumors: In rare cases, tumors in the mother can produce androgens, affecting fetal development.

Ambiguous genitalia in genetic males (XY chromosomes) can result from:

- Impaired Testicular Development: Genetic abnormalities or unknown factors can disrupt normal testicle development.
- Androgen Insensitivity Syndrome (AIS): Tissues fail to respond to androgens, preventing typical male genital development.
- 5-alpha-Reductase Deficiency: This condition impairs the conversion of testosterone to dihydrotestosterone, a potent androgen, affecting genital masculinization.
- Testicular Structural or Functional Issues: Problems with testosterone production or cellular androgen receptors can

also lead to ambiguous genitalia.

Ambiguous genitalia cases may have many cytogenetic abnormalities like 46XX virilized female, undervirilized 46XY male, and true hermaphrodite 46XY/46XX mosaics. Many syndromes like trisomy 18, mosaic turner 45XO/46XX, SRY gene and no SRY signals, Denys –Drash, Fraiser, WAGR and Robinoware are associated with ambiguous genitalia [2].

In some cases of ambiguous genitalia chimera with two different cell lines (46XX and 46XY) has been found. Also,a translocation between chromosome Y and chromosome 7 has been found [11]. Chimerismresults from the fusion of 2 different zygotes in a single embryo [12] whereas mosaicism results from a mitotic error in a single zygote. In a study mosaic turner genotype was found [13].

Disorders of sex development (DSDs), which can lead to ambiguous genitalia, are sometimes accompanied by other congenital anomalies. Examples include congenital adrenal hyperplasia (CAH), anorectal malformations, isolated cloacal anomalies, hypospadias, Prader-Willi syndrome, Klinefelter syndrome, and mosaic Turner syndrome.

The presence of ambiguous genitalia, typically evident at birth or shortly thereafter, creates significant emotional distress for families, making it both a medical and social urgency. Early and accurate diagnosis is crucial for appropriate management and gender assignment. Chromosomal studies, combined with ultrasonography, play a vital role in achieving this. This allows for timely surgical and hormonal interventions, enabling the child to develop a consistent gender identity. Therefore, understanding the importance of cytogenetic studies in the diagnosis and management of ambiguous genitalia is essential for healthcare professionals.

2. MATERIAL AND METHODS

This descriptive study was conducted at King George's Medical University (KGMU), UP, Lucknow, with ethical approval from the institution's review board (letter number 2083/Ethics/R.Cell-17). The research was a collaborative effort between the Anatomy Department's cytogenetic laboratory and the Pediatric Surgery Department.

A total of 24 children with ambiguous genitalia were included in the study .Patient screening occurred in the Pediatric Surgery outpatient department (OPD). Participants included individuals with a clinical diagnosis of ambiguous genitalia, as determined by pediatricians and pediatric surgeons, who provided informed consent. Patients who declined consent were excluded. A thorough medical history, focusing on factors influencing disorders of sex development (DSD), was collected. Peripheral blood samples were obtained and analyzed in the cytogenetic laboratory. Karyograms were generated and evaluated to determine chromosomal abnormalities.

Aim and objective-

This study aimed to determine the chromosomal karyotype in patients with ambiguous genitalia and to identify any co-occurring congenital anomalies

3. RESULTS

A total of 24 children with suspected ambiguous genitalia lying in the sampling frame were included in the study. After obtainingmedical and other relevant history the blood samples collected were subjected to karyotyping.

In this study of 24 patients with ambiguous genitalia, 12.5% (3 cases) exhibited co-occurring anomalies. These included isolated anorectal malformations in 8.3% (2 cases) and anorectalmalformation with Turner syndrome in 4.2% (1 case)

Table 1: Distribution of cases with other associated anomalies (n=24)

SN	Anomalies	No. of cases	Percentage
1-	Isolated Anorectal malformation	2	8.3
2-	Anorectal malformation with Turner’s syndrome	1	4.2
	Total	3	12.5

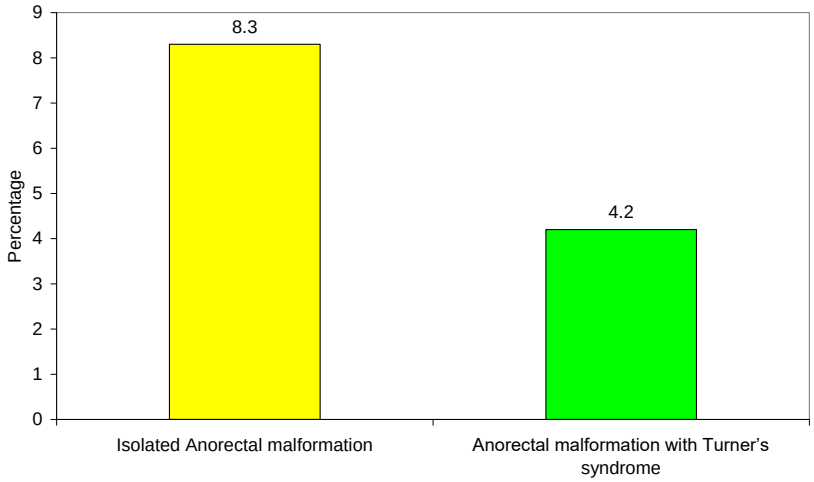

Fig1: Bar diagram showing associated anomalies encountered in the study population

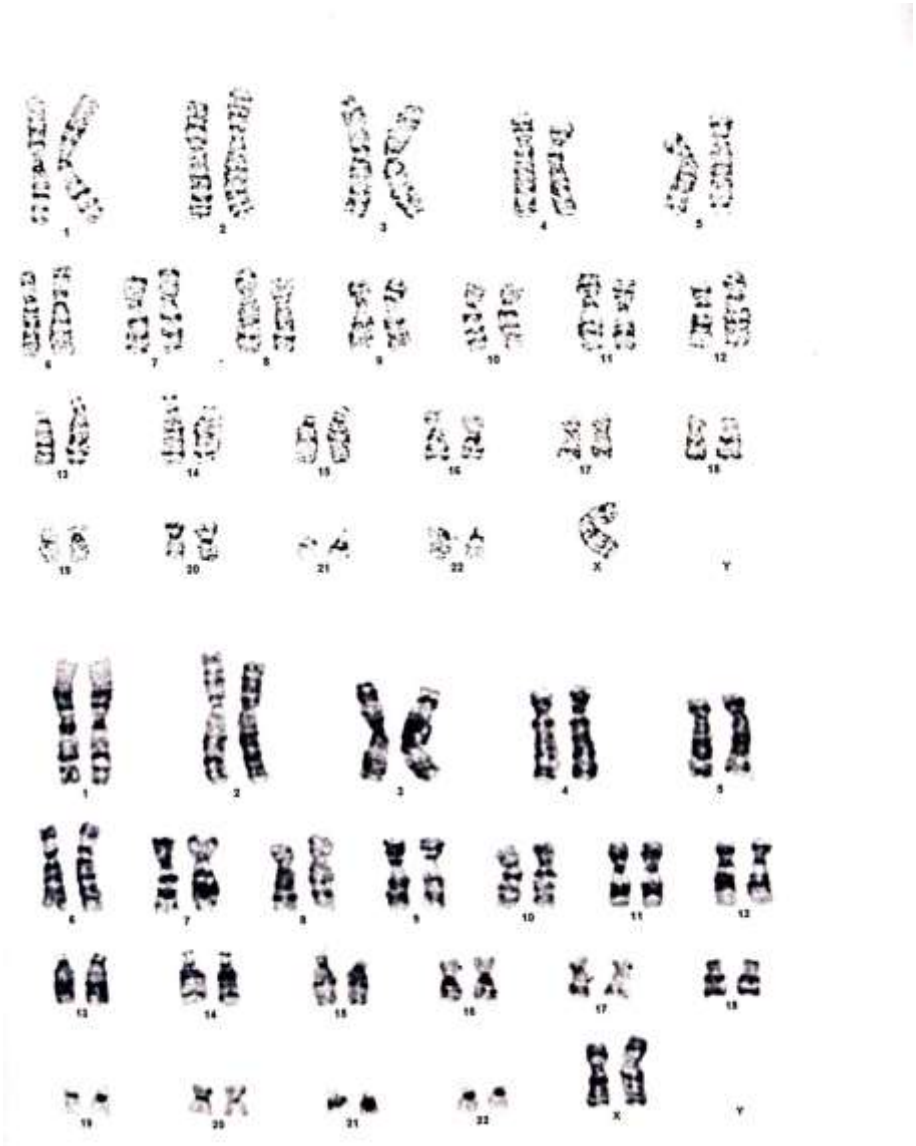

Fig. 2 45XO/46XX mosaic turner syndrome

4. DISCUSSION

Disorders of sexual development (DSD) encompass a group of congenital conditions associated with atypical development of internal and external genital structures. These conditions can be associated with variations in genes, developmental programming, and hormones. Affected individuals may be recognized at birth due to ambiguity of the external genitalia. Others may present later with postnatal virilization, delayed/absent puberty, or infertility.

In the present study, out of the total study group (24), associated anomalies (involving the gastrointestinal tract) were found in 3 (12.5%) cases, whereas those without any associated anomaly were found in 21 (87.5%) cases (Table 1, Figure 1). Thus, the variation in our study could be due to a small study population.

Heeley et al. performed a retrospective chart review of patients diagnosed with DSD conditions from 1995 to 2016 using ICD9 codes in Amarillo and found that the most common anomalies were cardiac anomalies in 28/128 (22%), skeletal anomalies in 19/128 (15%), and failure to thrive 19/128 (15%). Additional congenital anomalies were found in 53 out of 128 patients (41%) [16]. Our study was not consistent with above study.

Al-Jurayyan conducted a study on 81 patients from 1989-2008 in Saudi Arabia and found anorectal malformation in 2(7.14%) cases. Our study is nearly consistent with the given study slight variation might be due to large sample size and long duration of study period [15].

Al-Mulhim et al. (2010) performed a study in Saudi Arabia on 41 patient of which karyotype couldn't be determined in 6 patients. Eleven (34.4%) patients had extragenital anomalies. Our study is not in agreement with this study which may be due to different geographical region [14].

The phenotypic sex of a person depends on the type of gonad that develops in the embryo, a process that in itself is determined by the constitution or genetic inheritance of the individual, although the development of the gonads is different from that of any other organ, since they have the potential to differentiate into two functionally distinct organs, i.e., testes or ovaries [17, 18]. A person's sexual identity also include any behaviours that have sexual connotations, such as distinctive gestures and habits, speech patterns, leisure choices, and dream content, among many other factors that indicate an individual's sex. It is true that hormones have an impact on both internal and external genital development and differentiation. It has also been demonstrated that the embryo's brain can differentiate sexually, possibly via control mechanisms akin to those the external genitalia develop [19].

Sex development is achieved by the precise synergistic temporal-spatial expression of numerous activating and repressing factors. Deviations from this established developmental sequence can result in disorders of sex development. Investigations into the molecular basis of DSDs in patients have elucidated many genes and genetic regulatory mechanisms involved in this process [20].

5. CONCLUSION

DSD is associated with many other anomalies also like Congenital adrenal hyperplasia involving the adrenals .This is the most common associated anomaly . Others may include Anorectal malformations , Isolated cloacal anomalies, Hypospadias . Chromosomal anomalies like Prader Willi syndrome, Klinefelter's syndrome, Mosaic Turner syndrome etc may also be included.

In present study 12.5 % cases were associated with other anomalies of these 12.5% cases8.3 % had anomalies related to digestive system and 4.2 % had involvement of digestive system as well as chromosomal anomalies were also associated. Although data is not significant but still we found some association. This may be due to small sample size and short duration of study and the study need to be further continued for more significant results.

DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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