Clinical Characterization of Children and Adolescents with Disorders of Sex Development Attending a Tertiary Centre in the Western Cape, South Africa

By

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Declaration Page

I, Ewuraa Abena Owusuaa Manu, hereby declare that the work on which this dissertation is based is my original work, and acknowledgements have been indicated in situations where another person's work has been referenced or quoted.

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May the good God bless you all.

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Abbreviations

17-OH PROGESTERONE	- 17 hydroxy progesterone
AMH	- Anti Müllerian hormone
AT	- Adjusted Testosterone
CAH	- Congenital Adrenal Hyperplasia
CAIS	- Complete Androgen Insensitivity Syndrome
DSD	- Disorders (difference) in Sex Development
ESPE	- European Society of Paediatric Endocrinologists
FSH	- Follicular Stimulating Hormone
GSH	- Groote Schuur Hospital
HCG	- Human Chorionic Gonadotropin
LH	- Luteinizing Hormone
LWPES	- Lawson Wilkins Paediatric Endocrine Society
OT	- Ovotesticular
PAIS	- Partial Androgen Insensitivity Syndrome
QF-PCR	- Quantitative Fluorescence-Polymerase Chain Reaction
RCWMCH	- Red Cross War Memorial Children's Hospital

Abstract

Background: The objectives of our study were to describe the presentation, classification, and underlying causes, where possible, of Disorders of Sex Development (DSD) cases in a middle-income country in Africa.

Methods: This was a retrospective review of all DSD cases referred to a Paediatric Endocrine unit in a tertiary hospital in South Africa from January 2006 to December 2021. The biochemical data were adjusted based on the reference range applicable to the chronological age and chromosomal sex.

Results: Of the 139 patients analysed, 70 (50.4%) were 46, XY DSD, 46 (33.1%) were 46, XX DSD, and 23 (16.5%) were sex chromosome DSD. The mean adjusted testosterone (AT) at presentation did not differ between 46, XX DSD [AT: 0.4 (0.10-0.80)] and 46, XY DSD [AT: 0.4(0.10-1.05)]; p=0.76. Male sex was assigned at birth to 78 (67.2%) of both 46, XY, and 46, XX DSD groups. Of these, 73 (93.6 %) were assigned male gender of rearing. A precise diagnosis beyond a defect of androgen synthesis or action could not be made in 48 (68.6%) of the 46, XY DSD group. In contrast, 42 (91.3%) of the 46, XX DSD group had a precise diagnosis; 27 (64.3%) had ovotesticular DSD, 8 (19.0%) congenital adrenal hyperplasia (CAH), 5(12.0%) testicular DSD, and 2(4.7%) gonadal dysgeneses.

Conclusion: In our cohort, 46, XY DSD predominated. Concordance between the sex assigned at birth and the gender of rearing after evaluation was 79.1%. The mean AT did not discriminate between various DSD categories. Ovotesticular DSD was the most common diagnosis among 46, XX DSD, and the reasons for this need to be explored.

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RESEARCH ARTICLE

Clinical Characterization of Children and Adolescents with Disorders of Sex Development Attending a Tertiary Centre in the Western Cape, South Africa

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Short Title: Ovotesticular disease predominates in 46, XX DSD: A retrospective study from South Africa

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Abstract

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Methods: This was a retrospective review of all DSD cases referred to a Paediatric Endocrine unit in a tertiary hospital in South Africa from January 2006 to December 2021. The biochemical data were adjusted based on the reference range applicable to the chronological age and chromosomal sex.

Results: Of the 139 patients analysed, 70 (50.4%) were 46, XY DSD, 46 (33.1%) were 46, XX DSD, and 23 (16.5%) were sex chromosome DSD. The mean adjusted testosterone (AT) at presentation did not differ between 46, XX DSD [AT: 0.4 (0.10-0.80)] and 46, XY DSD [AT: 0.4(0.10-1.05)]; *p*=0.76. Male sex was assigned at birth to 78 (67.2%) of both 46, XY, and 46, XX DSD groups. Of these, 73 (93.6 %) were assigned male gender of rearing. A precise diagnosis beyond a defect of androgen synthesis or action could not be made in 48 (68.6%) of the 46, XY DSD group. In contrast, 42 (91.3%) of the 46, XX DSD group had a precise diagnosis; 27 (64.3%) had ovotesticular DSD, 8 (19.0%) congenital adrenal hyperplasia (CAH), 5(12.0%) testicular DSD, and 2(4.7%) gonadal dysgenesis.

Conclusion: In our cohort, 46, XY DSD predominated. Concordance between the sex assigned at birth and the gender of rearing after evaluation was 79.1%. The mean AT did not discriminate between various DSD categories. Ovotesticular DSD was the most common diagnosis among 46, XX DSD, and the reasons for this need to be explored.

Introduction

In 2006, the Lawson Wilkins Paediatric Endocrine Society (LWPES) and the European Society for Paediatric Endocrinology (ESPE) proposed a new nomenclature and classification for what had previously been termed true and pseudo-hermaphroditism, with descriptors including under virilised male or virilised female.[1] The proposed classification aimed to provide a generally accepted framework for reporting Intersex persons, to generate comparable and better data, and to inform the management of affected persons. The term Disorders of Sex Development (DSD) replaced hermaphroditism and intersex as diagnostic terms, although Intersex remains a term by which some persons with a DSD identify. Subsequently, the word 'Differences' has been proposed to replace 'Disorders'.[2] DSD describes any congenital condition resulting from atypical development of chromosomal, gonadal, and anatomical sex. DSD encompasses a heterogeneous group of conditions with varied phenotypic features and pathophysiology, manifesting as complete sex reversal (phenotype and genotype of opposite sex), atypical genitalia (not fully male or female), ambiguous genitalia (sex at birth not immediately evident) or as part of a syndrome affecting other organ systems.[3] Time of presentation ranges from birth to adulthood: atypical genitalia, genitalia not concordant with an antenatal scan or karyotype results, or Addisonian crisis in infancy, virilisation or precocious puberty in childhood, abnormal pubertal development in adolescence, and infertility in adulthood.[4,5]

The underlying diagnoses of DSDs are diverse. Without genetic testing and molecular facilities, the precise underlying diagnosis of many DSDs is difficult. However, an early presumptive diagnosis and well-thought-through medical plan are imperative to promote the physical and mental well-being of babies, children, and adolescents with a DSD. Of particular importance is the prevention of adrenal crisis in 21-hydroxylase deficiency (CAH), prevention or early detection of malignancy in undescended testes, improvement of long-term skeletal and cardiac health, and improved quality of life-related outcomes.[3,5,6]

The classification proposed by LWPES and ESPE recommends three main diagnostic categories: 46, XY DSD (formerly male pseudo hermaphroditism), 46, XX DSD (formerly female pseudo hermaphroditism), and sex chromosome DSD.[1] (Table 1)

TABLE 1: CURRENT CLASSIFICATION OF DSD[1]

46, XY DSD		46, XX DSD		Sex chromosome DSD
Disorders of testicular development	**Disorders of synthesis /action of androgens**	**Disorders of ovarian development**	**Disorders of synthesis /action of androgens**	
complete gonadal dysgenesis	defect in androgen biosynthesis (e.g., 3β-hydroxysteroid dehydrogenase 2 (HSD3B2), 5α-reductase 2 (SRD5A2) etc.)	ovotesticular DSD	Fetal Androgen excess (e.g., congenital adrenal hyperplasia	45, X (Turners syndrome and variants)
partial gonadal dysgenesis	LH receptor defect; Smith–Lemli–Opitz syndrome	testicular DSD (e.g., SRY+, duplication of SOX9 gene)	Fetoplacental aromatase deficiency oxidoreductase deficiency	47, XXY Klinefelter syndrome and variants
gonadal regression	insensitivity androgens e.g., CAIS	gonadal dysgenesis	Maternal virilising tumours (e.g., luteomas)	
ovotesticular DSD	disorders of AMH and AMH receptor (persistent Mullerian duct syndrome)		Androgenic drugs	45, X/46, XY Mixed gonadal dysgenesis ovotesticular DSD
Others (e.g., severe hypospadias, cloacal exstrophy)		Other (e.g., cloacal extrophy, vaginal atresia, MURCS, other syndromes)		

AMH, anti-Müllerian hormone; CAIS, complete androgen insensitivity syndrome; DSD, disorders of sex development; MURCS, Mullerian, renal, cervicothoracic somite abnormalities;

The 46, XY DSD, and 46, XX DSD groups are further subdivided into disorders of gonadal development (or sex determination) and disorders of androgen excess, synthesis, or action (or sex differentiation).[1] All categories include presentation with either typically female or male genitalia, atypical genitalia, or ambiguous genitalia (not absolutely female or male).

Sex chromosome DSD results from sex chromosome aneuploidy or sex chromosome mosaicism, giving rise to a disorder of gonadal development. Abnormal gonadal function affects sex differentiation, puberty, and fertility.[7] The category of Sex chromosome DSD includes Turner and Klinefelter Syndromes; as such, these patients present predominantly in childhood, adolescence, or adulthood, with problems related to the syndrome, delayed puberty, or infertility. Presentation in infancy with atypical or ambiguous genitalia is rare. Examples are also included in Table 1.[7]

The reported worldwide incidence of DSD is between 1: 4 500 and 1: 5 000 live births.[8] The reported incidence of genital anomalies in Europe has been reported from as low as 2 per 10,000 live births[9] in one study to 5.9 per 1,000 live births in another.[10] There is limited data from sub-Saharan Africa, with most studies being either observational or retrospective and limited by the absence of genetic and biomolecular testing.[11,12] The lack of comprehensive epidemiological data can be attributed to the diverse presentation, the rarity of the individual conditions, and the lack of available genetic testing in many parts of the world. In addition, traditional and cultural practices in certain parts of the world prevent persons with genital anomalies from accessing healthcare. [11,13,14]

Certain diagnostic categories appear to predominate in sub-Saharan Africa. For example, a study of DSD in a tertiary hospital in Kenya by Amolo *et al*,[11] reported that 46, XY DSD constituted 48.7% of their cohort. Similarly, a study from South Africa by Ganie *et al*. [15] reported that 46 XY DSD constituted 57.5% of their study population. In contrast, Abdullah *et al.* [16] in Khartoum, Sudan, and Sap *et al.* [12] in Yaoundé, Cameroon, found 46, XX DSD predominated in 60.5% and 55%, respectively, in observational retrospective reviews of DSD patients in their respective countries. The worldwide incidence of congenital adrenal hyperplasia (CAH) from most studies ranges from about 1:14,000 to 1:18,000 births. Almost 95% of CAH are caused by mutations in *CYP21A2*, an enzyme encoding for the adrenal steroid 21-hydroxylase (P450c21).[17] Data from parts of Europe and Australia have shown CAH to be the predominant diagnosis among their 46, XX DSD cohorts.[9,18] A study in Finland found CAH predominating in their 46, XX DSD category; however, Turner syndrome was the most common underlying diagnosis in that DSD cohort.[3] The predominance of certain causes of DSD in various parts of the world attests to the heterogeneity of this group of conditions.

The diagnosis of DSD warrants a multidisciplinary approach involving endocrinologists, urologists, radiologists, clinical geneticists, psychologists and/ or psychiatrists, and neonatologists, depending on the age of presentation.[19] Diagnosis and management can be challenging for clinicians worldwide on account of overlapping phenotypes, genetic heterogeneity, gender assignment, surgical management, and limited availability of genetic testing.[20] León *et al.* [20] propose a holistic algorithm that integrates traditional clinical

practices, including physical examination, biochemical analysis, imaging biopsy, and laparoscopy with molecular diagnosis. Molecular diagnosis and new emerging technologies, such as whole exome sequencing and next-generation genome mapping, have been used to help make a definitive underlying diagnosis.

In our unit, all suspected cases of DSDs have a karyotype ± Quantitative fluorescence-polymerase chain reaction (QF-PCR) performed at first contact. If gonads are not palpable on examination, an abdominopelvic ultrasound scan is performed to assess the presence of Müllerian structures. Luteinising hormone (LH), Follicular stimulating hormone (FSH), Anti Müllerian hormone (AMH), testosterone, and oestrogen levels are measured from birth till six months of age because of the mini puberty window. Those seen as neonates will most importantly have the adrenal androgens DHEAS (Dehydroepiandrosterone sulphate), 17-hydroxy progesterone (17OH P), cortisol, and electrolytes measured on day 3 of life. Those with hyponatremia, hyperkalaemia, and low cortisol are started on hydrocortisone and fludrocortisone while awaiting the results of DHEA and 17 OH P levels.

For a 46, XY karyotype, a Human Chorionic Gonadotropin (hCG) stimulation test is done to assess the presence and functionality of Leydig cells when the child presents after six months or the neonatal testosterone level is not conclusive. If the hCG stimulation test confirms testosterone synthesis and the AMH level is appropriate for age and sex, a laparoscopy is performed by the surgeons for biopsy and a possible orchidopexy as the testes are deemed functional per the results. However, if the hCG stimulation test fails, with low AMH results, a preliminary diagnosis of gonadal dysgenesis is made, and a laparoscopy will be performed for gonadectomy. The laparoscopic findings and histopathology report on the biopsied gonads are key in making the final underlying diagnosis.

For a patient of 46, XX karyotype without palpable gonads and with Müllerian structures, high 17-OH progesterone and DHEA-S, hyponatraemia, hyperkalaemia, and hypocortisolaemia are key in diagnosing CAH as the final diagnosis. In a patient who is 46, XX with palpable gonads, an AMH and HCG stimulation test assesses the presence and functionality of Sertoli and Leydig

cells. If the hCG stimulation test is positive and AMH is above the reference range for age and genetic sex, then, in our setting, it is assumed that functional testicular tissue is present with an underlying diagnosis of ovotesticular DSD. A laparoscopy is performed to confirm pelvic structures, and a gonadal biopsy is performed to ascertain the final diagnosis. The LH, FSH, oestrogen, and testosterone levels are only assessed within the window for mini puberty. Laboratory investigation for AMH and QF-PCR only became routinely available in our unit in recent years.

Based on observational data from our clinic and another study from South Africa,[15] we hypothesised that the majority of this cohort of DSD were likely to be 46, XY DSD, without a precise underlying diagnosis. We also hypothesised that most of our patients would demonstrate concordance between the sex assigned at birth and the gender of rearing after evaluation by our multidisciplinary team. Our objectives were, therefore, to describe the presentation, classification, and underlying defects, where possible, and follow-up of children and adolescents with DSD in a middle-income country and to determine the concordance between sex assigned at birth and gender of rearing.

Aims and Objectives

General Aim

To describe the spectrum of Disorders of Sex Development (DSD) encountered by the Paediatric and Adolescent Endocrine services of Red Cross War Memorial Children's Hospital (RCWMCH) and Groote Schuur Hospital (GSH).

Specific Objectives

1. To describe the characteristics of patients with DSD at presentation to the Paediatric and Adolescent Endocrine services at the RCWMCH or the GSH from 1 January 2006 until 31 December 2021 under the following:
    i. Phenotype, genotype, hormonal profile, imaging, gonadal biopsy
    ii. Diagnosis
    iii. Birth-assigned gender
    iv. Gender of rearing
    v. Surgery
2. To correlate the clinical characteristics of children with DSD who presented to the Paediatric Endocrine service at the RCWMCH and GSH from 1 January 2006 to 31 December 2021 with the biochemical, molecular, and cytogenetic findings.

3. To determine the outcome of the children with DSDs diagnosed at Paediatric Endocrine service at the RCWMCH and GSH from 1 January 2006 to 31 December 2021 based on the following interventions and subjective assessment:
    i. Gonadectomy
    ii. Hormone replacement
    iii. Gender experience
    iv. Ongoing healthcare

Materials and Methods

This study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, South Africa. Individual informed consent was waived due to the retrospective nature of this study while maintaining the anonymity of the participants. This study was executed based on good clinical practice guidelines, advised by the latest declaration of Helsinki.[21]

Sample size

A total of 139 folders were identified and analysed. The study included all folders with diagnoses meeting the case definition. A sample size was not calculated as this was a retrospective review of folders.

Sampling procedure

We reviewed the clinical records of patients attending the Paediatric and adolescent endocrine clinic at RCWMCH and GSH and the discharge summaries of the endocrine cases over the study period.

Using the case definition, all patients with a DSD were identified before enrolling on this study. A predesigned data extraction sheet was used to extract the demographic and clinical data, including the mode of presentation, description of the external genitalia, and presence of palpable gonads. We excluded patients referred with distal (anterior) hypospadias with bilaterally descended testes, as such patients usually were not evaluated as a DSD.[22] However, we included boys with proximal hypospadias and unilateral or bilateral cryptorchism who were referred to with a suspected DSD.[23,24]. We also excluded patients referred to the service with congenital anomalies of the genitalia that were not related to sex development (imperforate hymen, labial fusion). There were no cases of other congenital malformations of

the genitalia or of cloacal exstrophy, vaginal and/or uterine atresia recorded during the study period.

The QF-PCR, chromosome analyses, and biochemical data were collected from the National Health Laboratory Service (NHLS) database, a contemporaneous repository of biochemical, histology reports, and genetic data.

The QF-PCR analyses were performed at NHLS Cytogenetics at GSH. The test procedure is recorded as follows: QF-PCR (Elucigene QSTRplusv2) and the test targets highly polymorphic, chromosome-specific short tandem repeats (STR) markers on chromosomes 13, 18, 21, X, and Y. Informative markers can be diagnostic of the chromosomal copy number. The DNA was extracted from peripheral blood, followed by QF-PCR and capillary electrophoresis (ABI 3500 Genetic Analyser, Applied Biosystems). The chromosome copy number was determined by comparison of peak area ratios. The limitations of the QF-PCR are the test's inability to detect mosaicism or small segment imbalance for the chromosomes tested and detect abnormalities on the other chromosomes.

The chromosomal analyses at the NHLS cytogenetics laboratory at GSH were performed on 10 (or 30 if indicated for possible mosaicism) metaphase cells after culture as per standardised methods. The chromosome band resolution is approximately 500 g-bands. Chromosomal changes that may be clinically significant, such as subtle rearrangements or micro-deletions, may not be detected in some metaphase spreads.

LH, FSH, Testosterone, 17OHP, and AMH were measured on a Roche Cobas automatic analyser at the NHLS Laboratory at GSH. Different analysers were used if samples had been sent to another laboratory that did not use the Roche Cobas. The Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) mobile application database reference intervals were used where the laboratory did not provide paediatric reference ranges. This mobile application has been validated and published in international medical journals.[25] The

CALIPER mobile application requires the input of the laboratory equipment used in running the test, which was recorded in the laboratory reports.

Anti Mullerian Hormone (AMH), another useful parameter in assessing the presence of testicular Sertoli cells, was documented. A higher AMH concentration in the 46, XX DSD indicates the presence of functional testicular tissue. In contrast, a low level of AMH in the 46, XY DSD confers the possibility of dysgenetic gonads or anorchia.[30] The assay for measuring AMH was unavailable from the NHLS for most of the study period but was outsourced if necessary.

The biochemical data were adjusted for age and chromosomal sex. The adjusted biochemical data were calculated by determining the ratio between the recorded value and the upper reference range for the respective chronological age and chromosomal sex when applicable. For example, mean adjusted testosterone (AT) was the ratio of measured serum testosterone concentration at presentation divided by the upper limit of the reference range, applicable to the chronological age and chromosomal sex.

We performed an hCG stimulation test in older infants and children if testosterone synthesis had not been confirmed in early infancy. However, the hCG was not routinely available in our unit, and whichever preparation was available was used. The hCG is a double polypeptide hormone that shares a common LH subunit. The LH receptors stimulate testicular Leydig cells to secrete testosterone (and other androgens). The test, performed over four days, evaluates the presence and/or function of testicular Leydig cells. The basal testosterone level is taken on the first day of the procedure before a 2000 IU of hCG is injected subcutaneously. The hCG injection is given daily on the subsequent two days at the same time as day 1. On the fourth day, blood is sampled for a possible stimulated testosterone level. Testosterone synthesis by the gonads (positive test) was confirmed by a 2-3-fold increase in testosterone from baseline to D4 [28].

A physician with experience in foetal assessment and pelvic ultrasound in infants and young children performed ultrasound.

The paediatric urology team at RCWMCH performed surgery in most cases. The histopathology team processed the frozen section of a gonad biopsy by placing the specimens in a Tissue-tex compound on a piece of cork. The specimens were then mounted on a cryostat chuck and frozen in liquid nitrogen. The chuck with the frozen tissues were clamped into the specimen holder and orientated parallel to a microtome in the cryostat. The microtomes were used to trim up the specimen until the whole face of the specimen had been exposed and then cut up to a standard thickness of 7mm. The cut-up sections were gently picked up and placed on a slide. At room temperature, the frozen section adhered to the slide. The slides were then stained with a rapid Haematoxylin and Eosin stain. The residual tissues were then formalin-fixed overnight and paraffin-embedded for formal sections the next day.

The pertinent radiological findings were extracted from the clinical notes and/or the hospital repository. The radiological images and surgical data with their respective histopathology were also extracted if available.

Case definition:

All folders belonging to neonates with a diagnosis or assessment of:

I. An overt genital ambiguity
II. Apparent female genitalia, with an enlarged clitoris, posterior labial fusion, or an inguinal/labial mass
III. Apparent male genitalia with bilaterally undescended testes, micropenis, isolated perineal hypospadias, or mild hypospadias with undescended testis/testes
IV. A family history of DSD
V. A discordance between genital appearance and a prenatal karyotype

All folders of infants, children, and adolescents with the diagnosis or requiring assessment for:

I. Previously unrecognised genital ambiguity
II. Bilateral inguinal hernia in a female
III. Delayed or incomplete puberty
IV. Virilization in a female
V. Primary amenorrhea in individuals aged 16 years or older
VI. Gynaecomastia in a male
VII. Cyclical haematuria in a male
VIII. Congenital Adrenal Hyperplasia
IX. Sex chromosome DSD, e.g. Turners syndrome

Inclusion criteria

1. All patients referred to the service who were diagnosed with a DSD according to the 2006 classification during the study period.

Exclusion criteria

1. All patients referred to the service who did not have a diagnosis of DSD after evaluation
2. Distal hypospadias, imperforate hymen, labial fusion
3. 46 XY patients with 21 hydroxylase deficiency

Statistical analyses

Descriptive statistics (median [interquartile range]) were used to summarise the findings of the continuous variables, for example, age at presentation, based on non-normative data distribution. The Shapiro-Wilk test assessed the normality or otherwise of continuous variables. Standard non-parametric analytical method: The Kruskal–Wallis test was used to compare age at presentation and all the continuous variables of the biochemical data except the AT. The AT concentration was analysed with one-way ANOVA, as the values were normally

distributed. A 2x2 contingency table was used to analyse the continuous variables. The Fischer exact test was used to compare categorical data where the data set comprised five or fewer, and the Chi-square test was used for larger categorical data sets.

The data were analysed using Microsoft® Excel® 2019 MSO (Version 2211 Build 16.0.15831.20098), while the Shapiro-Wilk test, Kruskal –Wallis test, one-way ANOVA, Fischer exact test, and Chi-square test were performed with the aid of GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

Results

Diagnostic categories

A total of 139 patients were identified, and their respective medical records were analysed. Based on the 2006 LWPES and ESPE current classification for DSD, the classification groups were: 70 (50.4%) 46, XY DSD; 46 (33.1%) 46, XX DSD, and 23 (16.5%) sex chromosome DSD.

Baseline characteristics

The age at first presentation to the Endocrine unit for the 46, XY DSD, and 46, XX DSD groups varied from the first day of life to 189 months. Atypical genitalia were the reasons for referral in 89 (76.7%) of the combined 46, XY DSD, and 46, XX DSD cohorts, while the remainder were referred with posterior hypospadias with or without undescended testes, or gynaecomastia in a male, among others. Only 4 (3.4%) of the cohort had a family history of genital ambiguity.

In the 46, XY DSD group, 48 (68.6%) were referred with atypical genitalia, and 45 (64.3%) had palpable gonads. Also, 56 (80%) had hypospadias documented in the clinical notes, out of which 46 (82.1%) were documented as posterior hypospadias (penoscrotal/mid-scrotal/perineal). Eleven of the 46 (24%) 46, XY DSD with posterior hypospadias had no palpable gonads documented. Although 11 (15.7%) of the 46, XY DSDs were described as having normal-appearing external genitalia, we noted that they had no description of hypospadias documented, or had no palpable gonads. In the 46, XX DSD group, 41 (89.1%) were referred with atypical genitalia, and 19 (46.3%) had palpable gonads.

Atypical genitalia were more common in the 46, XY DSD group compared to the 46, XX DSD group. Gonads were palpable in 45 (64.3%) of 46, XY DSD, and 19 (41.3%) of 46, XX DSD. Records do not reveal if one or both gonads were palpable. Distal (glandular or sub-coronal) hypospadias were more common in the 46, XX DSD than the 46, XY groups. This was because data on distal hypospadias with palpable gonads were not included. (See Table 2)

Table 2: Baseline characteristics of patients

Variables	46, XY DSD (*N*=70)	46, XX DSD (*N*=46)	*p*-value
Age at 1st presentation (months)			
Median (IQR)	5.50(2.88-13.0)	3.50 (0.93-28.50)	0.09
Presenting complaints			
Atypical genitalia	48 (68.6%)	41 (89.1%)	**0.010**
Hypospadias (±undescended testes)	18 (25.7%)	2 (4.3%)	**0.003**
Gynecomastia	0 (0.0%)	2 (4.3%)	0.16
Others [a]	4 (5.7%)	1 (2.2%)	0.65
Examination findings			
Palpable gonads	45 (64.3%)	19 (46.3%)	**0.015**
External genitalia (Prader scale)			
Normal appearing / typical female external genitalia	1 (1.4%)	0 (0.0%)	1.00
Prader I	0 (0.0%)	2 (4.3%)	
Prader II	1 (1.4%)	3 (6.5%)	0.30
Prader III	16 (22.9%)	22 (47.8%)	**0.008**
Prader IV	8 (11.4%)	12 (26.1%)	**0.041**
Prader V	33 (47.1%)	6 (13.0%)	**0.0001**
Normal appearing /typical male external genitalia (Empty scrotum/hypospadias not documented)	11 (15.7%)	1 (2.2%)	**0.019**
Hypospadias[26] (documented)			
Posterior hypospadias (Peno-scrotal, Scrotal, Perineal)	46 (82.1%)	27 (73.0%)	0.44
Middle (distal penile, midshaft)	10	4 (10.8%)	0.56
Distal (sub-coronal/glandular)	0 (0.0%)	5 (13.5%)	**0.009**
Repaired hypospadias before presentation	0 (0.0%)	1 (2.7%)	0.38
Total (n)	56 (100%)	37 (100%)	
Family history of genital ambiguity	4 (5.7%)	0 (0.0%)	0.15

Others were evaluated for genetic abnormalities, testes in hernia sac, and empty scrotal sac.

The group of sex chromosome DSD constituted 23 (16.5%) of our cohort. The median age at presentation was 40.0 (24.0-111.0) months. Within this group, 3 (13.0%) were referred with atypical genitalia and palpable gonads, 10 (43.5%) with confirmed Turner syndrome, and 3 (13.0%) with the diagnosis of Klinefelter syndrome were referred from the genetic service for endocrine assessment and follow-up. The remainder were referred with short stature and delayed puberty 4 (17.4%), atypical male genitalia 1 (4.3%), and gynecomastia 2 (8.7%). The patients with short stature and delayed puberty all had the karyotype 45, X0 (*N*=4).

Biochemical and radiological features

Patients in the cohort did not all have the same set of investigations, as these were chosen according to the clinical presentation, including age at presentation. In addition, some tests

were not available at the time of presentation. In some cases, the results were not documented or available. For example, 21/70 (30%) of children with 46 XY DSD did not have FSH and LH tested because they were older than six months and beyond mini-puberty. Mini puberty describes the transient sex-specific activation of the hypothalamic-pituitary-gonadal axis, leading to a rise of LH, FSH, oestradiol, and testosterone. It occurs during the first 3-6 months of life in both sexes.[27]

Although the indication for measuring AMH differs for the two main DSDs, we noted that the results are not different in 46, XY DSD compared to 46, XX DSD.

When comparing the biochemical and radiological features among 46, XY DSD, and 46, XX DSD groups, as expected, when it was looked for, the SRY marker was present in 13/14 (93%) of the 46, XY DSD group compared with only 1/35 (2.3%) of the 46 XX DSD group (p=0.001), while sonographic evidence of Müllerian structures was seen in only 5/61 (8%) of the 46, XY DSD group compared with 22/44 (50%) of the 46, XX DSD group (p<0.001). Also, as expected, the HCG stimulation test was positive in 83.9% of the 46, XY DSD group, compared with 78.6% of the 46, XX DSD group (p= 0.048).

In contrast, the mean AT and anti-Mullerian hormone (AMH) concentrations did not differ between the 46, XY DSD, and the 46, XX DSD groups, respectively. (p=0.76; p=0.32). (Table 3)

In the 46, XX group, the mean baseline AT in patients with ovotesticular DSD was 2.88 and 1.18 in patients with CAH. However, the mean AT also did not differ significantly between the groups. (p=0.65).

Table 3. Comparison of biochemical and radiological features

Variables	**46, XY DSD (*N*=70)**	**46, XX DSD (*N*=46)**	***p*-value**
Baseline adjusted testosterone *			0.76
Mean	2.44	1.96	
SD	7.51	7.91	
SEM	1.03	1.24	
N	53	41	
Adjusted Anti-Mullerian Hormone (AMH) concentration			0.17
Median(IQR)	0.13 (0.10-1.09)	0.62 (0.12-8.0)	
N	14	15	
Adjusted 17 OH progesterone			0.38
Median (IQR)	0.61(0.41-1.20)	1.74(0.87-11.02)	
N	15	30	
Adjusted LH			0.31
Median (IQR)	0.33 (0.08-2.50)	0.08 (0.04-0.91)	
N	49	30	
Adjusted FSH			
Median (IQR)	0.3 (0.50-0.53)	0.99 (0.26-9.79)	0.05
N	49	30	
HCG stimulation test			
Positive	26 (83.9%)	22 (78.6%)	0.60
N	31	28	
Müllerian structures on ultrasound			
Present	5 (8.2%)	22 (50.0%)	**<0.001**
N	61	44	
Presence of SRY marker			
Positive	13 (93.0%)	1 (3.0%)	**0.001**
N	14	35	

*The biochemical result values were adjusted for the patient's age and karyotype reference ranges.

The AT in the sex chromosome DSD group was 0.26 (0.10-0.54), and the median adjusted AMH concentration was 0.19 (0.10-3.2), which did not differ from the remaining groups. Two of the sex chromosome DSD had a positive HCG stimulation test; both had mixed gonadal dysgenesis as the final diagnosis. Müllerian structures were demonstrated on pelvic ultrasound in 16/19 (84.2%) patients with sex chromosome DSD.

Surgical procedures and histology reports

Surgical procedures were undertaken in 58/70 (82.8%) patients with 46, XY DSD, and 39/46 (84.8%) patients with XX DSD. Thirty out of 70 (42.9%) patients in the 46 XY DSD group had a hypospadias repair. Laparoscopic gonadal biopsy was carried out in 36/46 (78.3%) of patients with 46, XX DSD compared with 16 (23%) of patients with 46, XY DSD.

On comparing the surgical procedures and histology reports of patients with 46, XY DSD with those with 46, XX DSD, we observed that orchidopexy, with or without a biopsy of the gonads, and hypospadias repair with or without orchidopexy, were performed in the 41/70 (58.6%) patients with 46, XY DSD versus 20/46 (43.5%) of those with 46, XX DSD, (p <0.001). Biopsies of the gonads with or without hypospadias repair were performed in 17/70 (24.3%) of patients with 46, XY DSD, compared with 36/46 (78.3%) patients with 46, XX DSD (p <0.001).

Among the 16 histology reports for the 46 XY DSD group, 5 (31.3%) were bilateral dysgenetic testicular tissue. Two dysgenetic testicular tissues were intra-abdominal with normal appearing Leydig cells and, functional as confirmed by the HCG stimulation test,[31,32], orchidopexy was performed. Two were lodged in the labioscrotal folds and found to be functional testicular tissues after a positive HCG stimulation test. The last dysgenetic testicular tissue had functional tissue with an in-situ germ cell neoplasm; gonadectomy was performed for the neoplastic gonad, while orchidopexy was performed for the functional gonad.

Of the biopsies performed in the 46, XX DSD group, 14/36 (39.0%) revealed the presence of ovarian and testicular tissue, and 6/36 (16.7%) revealed bilateral dysgenetic testicular tissue without any ovarian tissue, while none were neoplastic. Four of these dysgenetic gonads were intra-abdominal and functional, as confirmed by the HCG stimulation, and underwent orchidopexy, while the remaining two were functional and in the labioscrotal folds. These were left in situ, and the children were assigned to the male gender of rearing.

At laparoscopy, absent gonads were documented in 3/58 (5.2%) patients with 46 XY DSD, while none of the 46 XX DSD who had surgery documented an absent gonad (p=0.015). (See Table 4)

Table 4. Comparison of surgical procedures and histology reports between 46, XY DSD and 46, XX DSD

Variables	46, XY DSD [*N*=70]	46, XX DSD [*N*=46]	*p*-value
Surgical procedures			
Hypospadias repair± orchidopexy	30 (51.7%)	0 (0.0%)	**<0.001**
Biopsy	6 (10.3%)	16 (41.0%)	**<0.001**
Biopsy ±hypospadias repair	11 (19.0%)	20 (51.2%)	**<0.001**
Orchidopexy± biopsy	11 (19.0%)	0 (0.0%)	**<0.001**
Vaginoplasty	0 (0.0%)	1 (2.6%)	0.40
Clitoroplasty	0 (0.0%)	1 (2.6)	0.40
Metoidioplasty	0 (0.0%)	1 (2.6%)	0.40
N	58 (100%)	39 (100%)	
Histology report			
Ovotesticular tissues ± dysgenetic testicular tissues	2 (12.5%)	14 (38.9%)	0.057
Bilateral dysgenetic testicular tissues	4 (25.0%)	6 (16.7%)	0.46
Dysgenetic testicular tissue + germ cell neoplasia in situ	1 (6.2%)	0 (0.0%)	0.31
Dysgenetic testicular tissue + viable ovarian tissue	0 (0.0%)	5 (13.9%)	0.31
Dysgenetic testicular tissue + viable testicular tissue	1 (6.2%)	0 (0.0%)	0.31
Streak gonads+ dysgenetic testicular/ovotesticular tissue	0 (0.0%)	2 (5.5%)	1.00
Testicular tissue + dysgenetic testicular tissue	1 (6.2%)	0 (0.0%)	0.31
Ovarian tissue only	0 (0.0%)	5 (13.9%)	0.31
Testicular tissue only	3 (18.8%)	3 (8.3%)	0.36
Normal immature testes	1 (6.2%)	0 (0.0%)	0.31
Dysgenetic gonads + Müllerian remnants	3 (18.8%)	1 (2.8%)	0.08
N	16 (100%)	36 (100%)	

Gonadal biopsies were performed in 5/23 (21.7%) patients with sex chromosome DSD, with 2 demonstrating extensive fibrosis and hyalinisation of the seminiferous tubules associated with interstitial hyperplasia. These were consistent with Klinefelter syndrome and confirmed by karyotype analysis. Dysgenetic testicular gonads with a streak gonad were confirmed histologically in the remaining three patients.

Final underlying diagnosis

Only (87/139) 62.6% of the cohort had a definitive underlying diagnosis. The final diagnosis was based on a review of the clinical and radiological features, biochemical data, and, in some cases, histology. Patients in the 46, XX DSD group were more likely to have a definitive diagnosis; 91.3% 95% CI [0.79, 0.98], compared with 31.4% 95% CI [0.21, 0.44] in the 46, XY DSD group (p<0.001).

Gonadal dysgenesis was more likely to be diagnosed in the 46, XY DSD group (14/70, 20% (95% CI [0.12, 0.31]) than) in the 46, XX DSD group (2/46, 4.3% (95% CI [0.01 to 0.15]); (p=0.034).

Ovotesticular DSD occurred more frequently in patients with 46, XX DSD; 27/46, 58.7% 95% CI [0.43, 0.73] than the 46, XY DSD 4/70, 5.7% (95% CI [0.02, 0.14]) group; (p<0.001). (See Table 5)

Table 5: Final diagnosis of DSD based on clinical and radiological features and biochemical data

Variable	**46, XY DSD (N=70)**	**46, XX DSD (N=46)**	***p*-value**
Diagnosis			
Definitive diagnosis	22 (31)	42 (91)	**<0.001**
Final underlying diagnosis			
Disorders of Sex determination			
	46, XY DSD	**46, XX DSD**	***p*-value**
Gonadal Dysgenesis *Campomelic dysplasia **Lenz microphthalmia	14 (64.0%) 1 (4.5%) 1 (4.5%)	2 (4.8%) - 0 (0.0%)	**0.034**
Ovotesticular DSD	4 (18.0%)	27 (64.2%)	**<0.001**
Testicular DSD	N/A	5 (12.0%)	
Disorders of sex differentiation			
	46, XY DSD	**46, XX DSD**	***p*-value**
Disorders of androgen excess • Congenital adrenal hyperplasia Disorders of androgen synthesis • Other (incl. 5α-reductase def, Leydig cell hypoplasia)	 0 (0.0%)	 8 (19.0%)	**<0.001**
Disorders of androgen action CAIS[1] PAIS[2]	 1 (4.5%) 1 (4.5%)	 0 (0.0%) 0 (0.0%)	

[1]-Complete androgen insensitivity syndrome [2]-Partial androgen insensitivity syndrome

15/23 (65.2%) of the sex chromosome DSD group had a definitive diagnosis of Turner syndrome, while 4/23(17.4%) had a definitive diagnosis of Klinefelter syndrome. The remaining 4/23 (17.4%) were diagnosed with mixed gonadal dysgenesis (45, X/ 46, XY).

Of the 48/70 46, XY DSD group with no definitive diagnosis, 35 (73%) did not have a complete biochemical dataset and 6/48 (12.5%) remained under investigation, at the time of our data

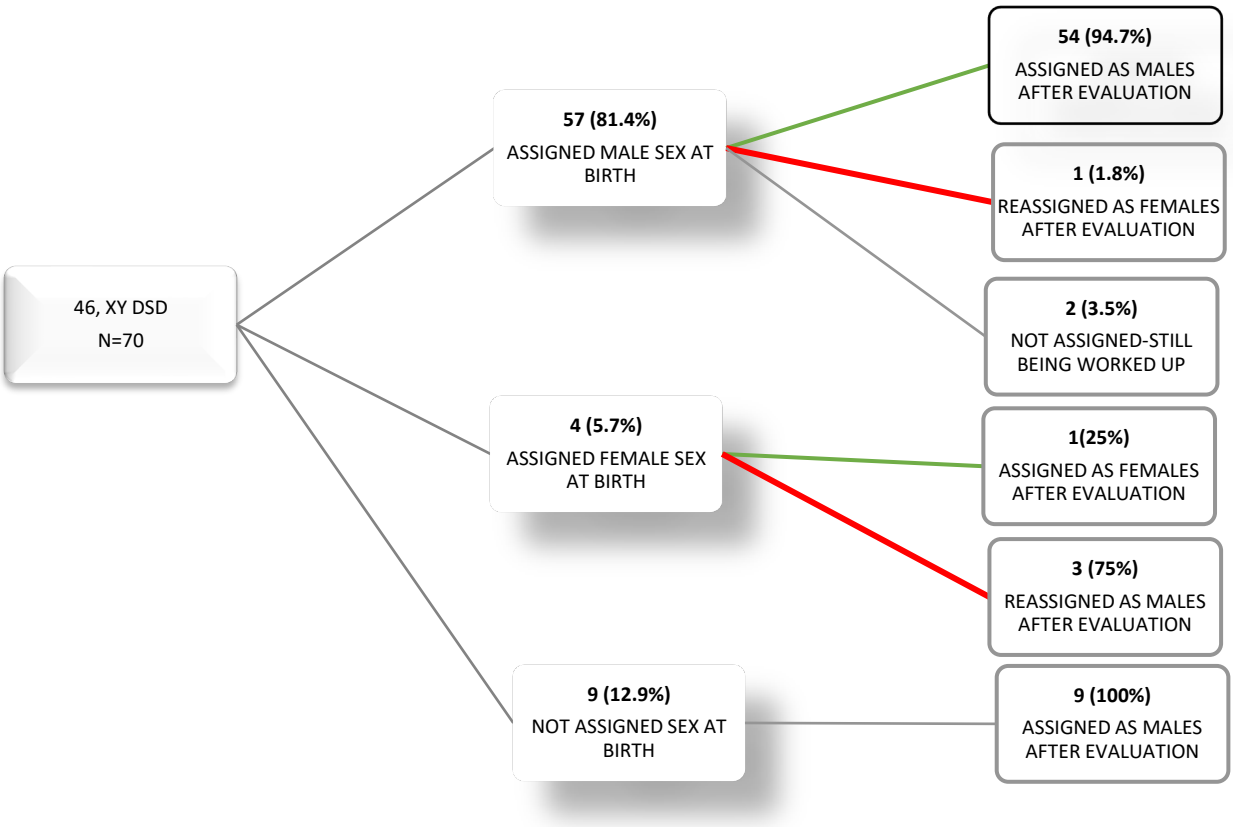
collection. The biochemical data was incomplete mainly because of the age at first presentation, after the mini puberty window, the unavailability of the AMH test, and possibly missing data.

Concordance between the sex assigned at birth and the gender of rearing assigned after evaluation.

The sex assigned at birth is usually based on the visual appearance of the external genitalia, performed by the staff at the health facility where the baby is delivered or by the parents and/or relatives of the baby, or on the appearance of the antenatal scan.

The final decision on the gender of rearing after an evaluation is multifaceted and based on the 2006 consensus statement on the management of DSD.[33] The gender of rearing for our cohort was decided upon after evaluation and discussion with the patient's parents and/or relatives. The patients' classification (i.e., 46, XY DSD, 46, XX DSD or sex chromosome DSD), gonadal function and histology, and, where possible, the definitive diagnosis were considered. If they were predictable, the anticipated pubertal development, future gender identity and role, and probable fertility options were also considered to help determine the gender of rearing. For older patients, gender identity plays a significant role in this decision-making. A psychiatrist was asked to assist with the assessment of the gender identity of some of these patients. The older patients underwent counselling sessions with the psychiatric/psychological team to ascertain their acceptance or not of the assigned gender of rearing.[32]

When comparing the sex assigned at birth with the gender of rearing in the 46, XY DSD group, 55/70 (78.8%) exhibited concordance. (shown in Fig. 1)

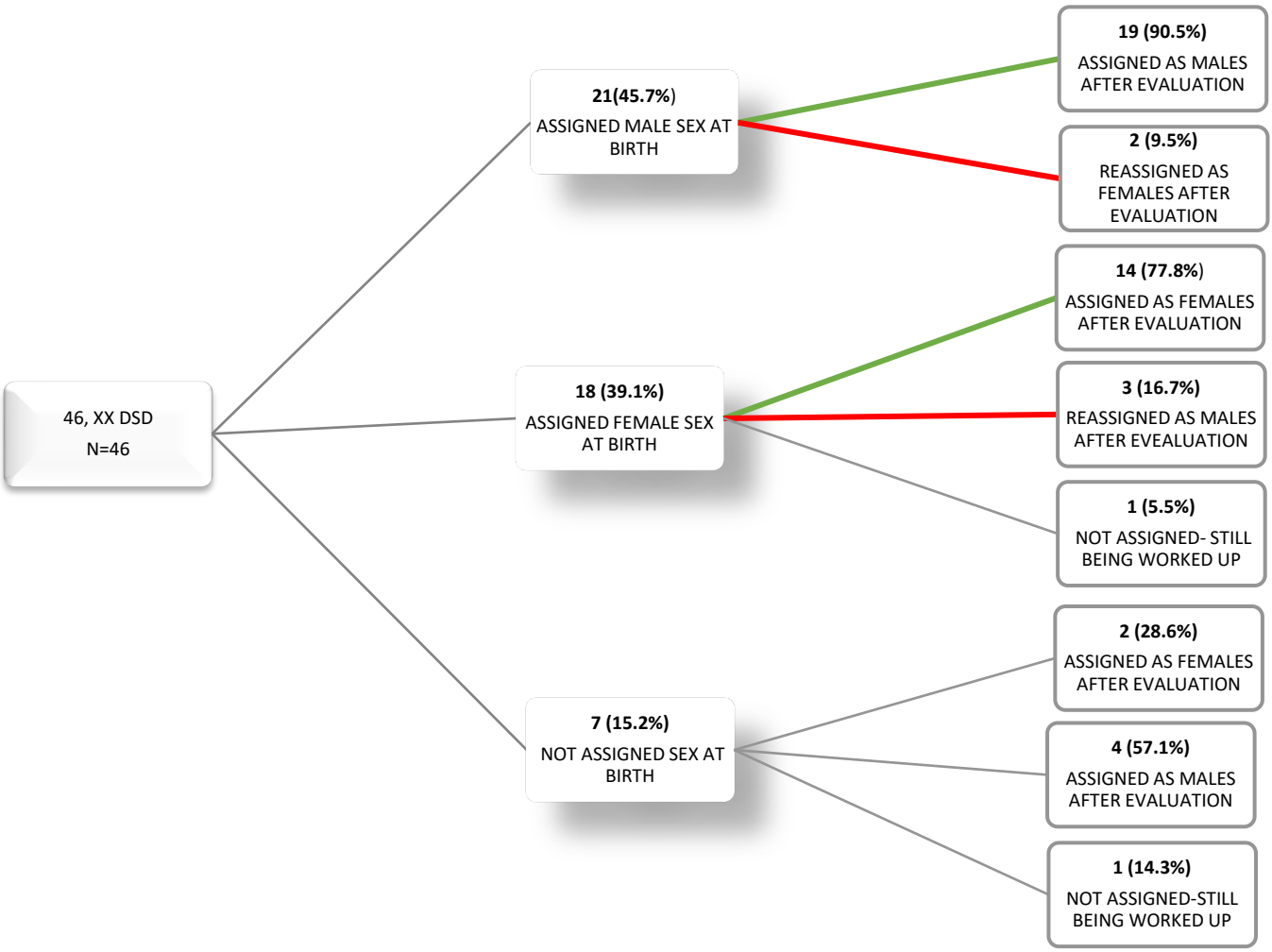


* Green lines show concordance between the sex assigned at birth and the assigned gender of rearing after evaluation

* Red lines show discordance between the sex assigned at birth and the assigned gender of rearing after evaluation

Fig. 1. Diagram showing the concordance between sex assigned at birth and assigned gender of rearing after evaluation in patients with a 46, XY DSD.

When comparing the sex assigned at birth with the gender of rearing in the 46, XX DSD group, 33/46 (71.7%) demonstrated concordance. (shown in Fig. 2).



* Green lines show concordance between the sex assigned at birth and the assigned gender of rearing after evaluation

* Red lines show discordance between the sex assigned at birth and the assigned gender of rearing after evaluation

Fig. 2. Diagram showing the concordance between sex assigned at birth and assigned gender of rearing after evaluation in patients with a 46, XX DSD.

In patients with sex chromosome DSD, 7/23 (30.4%) were assigned male sex at birth, and after evaluation, all were assigned male gender of rearing. Also, 16/23 (69.6%) were assigned

female sex at birth, of whom 15/16 (93.8%) were assigned female gender of rearing after evaluation, and 1/16 (6.2%) were reassigned male. The overall gender concordance for the sex chromosome DSD was 22/23 (95.7%).

Of the entire cohort, 89/139 (64.0%) remain in the care of the Paediatric Endocrine service and are either on hormone replacement treatment, being followed up annually, or booked for a follow-up at puberty. Patients lost to follow-up in this cohort constituted 41/139 (29.5%): 19 (46.3%) are from the 46, XY DSD cohort, 14 (34.1%) are 46, XX DSD cohort, and 8 (19.5%) are sex chromosome DSD. Nine (6.5%) have been discharged from the Paediatric Endocrine service.

Discussion

In this cohort, DSDs were categorised as 46, XY DSD (50.4%), followed by 46, XX DSD (33.1%), and sex chromosome DSD (16.5%). The overall concordance for sex assigned at birth and gender of rearing was 78.8% for the 46, XY DSD and 71.7% for the 46, XX DSD. Among the 46, XX DSD group, 27/42 (64.3%) had a final diagnosis of histologically proven ovotesticular DSD, whereas 15/23 (65.2%) of the sex chromosome DSD had Turner Syndrome. Our data showed that mean baseline AT concentration does not discriminate between the various diagnostic categories of DSD. Also, as expected, we demonstrated that a positive HCG stimulation test is more likely to occur in the 46 XY DSD group than in the remaining DSD groups. As predictable, it was also more likely for the 46, XY DSD group than the 46, XX DSD to undergo hypospadias repair with/without an orchidopexy, whereas a gonadal biopsy was done most frequently in the 46, XX DSD group, compared with the rest of the DSD groups.

Consistent with other studies from Africa, for example, Ganie *et al.* [15] in South Africa and Mazen *et al.* [34] in Egypt, the majority of our cohort was 46, XY DSD. In other studies outside of Africa, Beck *et al.* [35] from Brazil and Jahagirdar *et al.* [36] from India 46, XY DSD predominates in their cohorts. However, some studies emanating from the Republic of Cameroon and Sudan have shown that 46, XX DSD predominate in their cohorts.[12,37] Although consanguinity has been linked to a higher incidence of DSD in general, it remains unclear why some diagnostic categories occur more commonly in certain parts of the world.[38] The predominance of 46, XY DSD in some cohorts and our cohort may reflect a lower threshold in referring hypospadias to a Paediatric Endocrinology service.[39]

Equally, to other studies, 68.6% of our cohort of 46 XY DSD had no definitive final underlying diagnosis besides a presumptive disorder of androgen synthesis or action.[3,13,15,40] This is due to the unavailability of molecular tests, including mutational analysis and dihydrotestosterone assay, which are consistent with studies from South Africa and Egypt, where these factors were listed as limitations.[15,41]

There are several studies indicating that black South Africans with 46, XX DSD have ovotesticular DSD as the predominant underlying diagnosis, but the reason for this has so far been elusive.[15,42–44],[unpublished data] Ovotesticular (OT) DSD is a condition in which a child is born with both testicular tissue (that possesses variable fertility potential within seminiferous tubules) and ovarian tissue (with primordial follicles), co-existing either in the same gonad or independently.[45,46] The pathognomonic histologic feature of OT DSD is the presence of seminiferous tubules and ovarian follicles or oocytes or stroma, representing testicular and ovarian tissue, respectively, in the same patient. There is a wide spectrum of phenotypic presentation, including normal male external genitalia and varied degrees of atypical genitalia. Untreated cases with both functional ovaries and testes produce sex steroids at puberty, leading to breast enlargement and phallic growth [46]. In our cohort, 34/42 (81%) patients with 46, XX DSD had disorders of ovarian development; 27/34 (79%) had OT DSD. Similarly, a recent study from Kwa-Zulu Natal (KZN), South Africa, showed 76/114 (67%) patients with 46, XX DSD had disorders of sex determination (ovarian development); 69/76 (91%) had OT DSD.[42] CAH was diagnosed in 27/114 (24%) patients with 46, XX DSD in KZN, compared to 8/42 (19%) patients from this category in our cohort. However, none of our 46, XY or sex chromosome DSD patients who were biopsied demonstrated concurrent ovarian and testicular tissue. This is most likely because our cohort is smaller or possibly because we were more likely to have biopsied a person with 46, XX karyotype. This latter seems unlikely, but we do not have the data to confirm that no patients with 46, XY karyotype demonstrated ovarian function later in life. Given that both studies from South Africa have shown OT DSD to be the most prevalent diagnosis in patients with 46, XX karyotype, we agree with Ganie et al.[42] review that South Africans are presenting with 46, XX DSD should be investigated for OT DSD. However, CAH must be ruled out first.

Yet, in other parts of Africa, for example, Cameroon, CAH was the most common underlying diagnosis of their 46, XX DSD cohort.[12] While CAH was not the most common underlying diagnosis in our 46, XX DSD cohort, it was more likely for a 46, XX DSD to have CAH than 46, XY DSD. This is because 21 hydroxylase deficiency, the most common form of CAH, causes genital anomalies only in girls.[17] Disorders of androgen synthesis occurring as a result of enzymatic defects in the steroidogenesis pathway, e.g., 17-Hyroxylase/17,20-lyase (P450c17),

cause under-virilisation ranging from hypospadias to female-appearing genitalia. These other CAH forms are rare compared to the 21 hydroxylase deficiency.[47]

The biochemical data from our cohort were adjusted for the reference age and chromosomal sex for comparison across the age groups. From our results, the AT did not separate patients into various categories of DSD. A significant contributor is the age of presentation, as there is an overlap of testosterone concentrations among males and females before puberty.[19,48,49] Abdelghaffar *et al.* [50] from Egypt have demonstrated AMH to be a reliable marker to ascertain the presence and function of testes in infants and prepubertal children. Still, the study did not compare the AMH concentrations between 46, XY DSD, and 46, XX DSD. In our study, the adjusted AMH, limited by the small sample size, could not differentiate the various DSD categories. However, a comparison is not useful due to the different indications for using the test in 46, XY, and 46, XX DSD. As such, it is not a useful test to differentiate 46, XY DSD from 46, XX DSD. As expected, the presence of the SRY marker was more likely in the 46, XY DSD cohort than with the 46, XX DSD, as the SRY gene is needed for testicular development, and a mutation in this gene is reported to account for 10-15% of 46, XY DSD gonadal dysgenesis.[51]

In our cohort, 83.6% underwent a surgical procedure, mainly laparoscopy and biopsies of the gonads, to support the final diagnosis. In contrast, Amolo *et al.* [11] from Kenya found that fewer than half of their cohort had either laparoscopy or laparotomy, and 28% underwent biopsies of the gonads. One patient from our 46, XX DSD group with an underlying final diagnosis of ovotesticular disease underwent metoidioplasty (surgical creation of a penis from the clitoris) as a result of gender dysphoria. The patient was assigned female at birth and reared as female but experienced gender dysphoria as an adolescent. Two patients had vaginoplasty and clitoroplasty each. The vaginoplasty was performed for a patient with 46, XX DSD, with an underlying diagnosis of CAH at age 12 years. In contrast, vaginoplasty was performed for 46, XX DSD with ovotesticular DSD at puberty. This is different from other studies where many had genital reconstructive surgeries, for example, clitoroplasty and vaginoplasty at diagnosis.[13,52] Genital reconstructive surgery has evolved to prioritise the functional outcome over the cosmesis.[53,54]

Gender dysphoria, previously termed gender identity disorder, has been reported in the literature to have an overall prevalence of 15% (95% CI [13–17%]) among adolescents and adults with DSD.[55] Gender orientation is thought to be influenced by the androgen milieu in early life and not the presence or absence of the 'Y chromosome'.[55,56] This is evidenced by the low prevalence of gender dysphoria among those with 46, XX DSD CAH who are extremely virilised (Prader V) at diagnosis and raised as males. A low prevalence of gender dysphoria is also seen among 46, XY DSD with a final diagnosis of complete androgen insensitivity syndrome (CAIS) or complete gonadal dysgenesis reared as females.[55] In our study, only one patient (1/27, 4%) with a final diagnosis of ovotesticular DSD encountered gender dysphoria, as compared with the study from KZN, where 8/64 (12.5%) subjects had gender dysphoria. This may result from changing practices concerning assigning gender of rearing and early surgery over the two periods.

The median ages for the first presentation for our cohort are consistent with other studies, including 5.50 (2.88-13.0), 3.50 (0.93-28.50), and 40.0 (24.0-111.0) months for the 46, XY DSD; 46, XX DSD; and sex chromosome DSD group, respectively.[11,57] In contradistinction, a study by Sap *et al.*[12] from Cameroon found an older median age of 5.11 (1.76–6.73) years, 2.35 (0.19–6.43) years, and mean age of 13.73 ± 3.63 years for the 46, XY DSD; 46, XX DSD; and sex chromosome DSD group, respectively. The late presentation in some studies may be due to the lack of awareness,[11] cultural and traditional beliefs,[14] and religious or social beliefs,[58] *inter alia*. Compared with healthy controls, late presentation is also associated with impaired social and emotional function.[59] A significant number of our patients with 46, XY DSD presented with either hypospadias with or without undescended testes or with atypical male genitalia. This is consistent with a Turkish study in which almost all (93.4%) of the 46, XY DSD had hypospadias with or without undescended testes.[60] This is largely explained by the critical importance of testosterone and dihydrotestosterone in external male masculinisation, and where these are deficient, hypospadias and undescended testes tend to occur.[7,61]

There was a small subgroup of 8.3% of our entire cohort, for whom no gender could be assigned. Of these, 81% were assigned as males after evaluation, similar to most DSD studies.[11,13,16] Among the sex chromosome DSD, there was 95.7% gender concordance

between the sex at birth and the assigned gender of rearing after evaluation, which is explained by the lack of genital ambiguity in this subgroup of DSD.[35,62]

Our study was a retrospective review of folders, limiting our ability to verify the clinical data. The unavailability of an assay for measuring AMH, dihydrotestosterone, molecular studies, and incomplete data limited our ability to confirm a more precise diagnosis in the majority of the 46, XY DSD, which accords with most studies from Africa.[15,41] Also, gender satisfaction could not be evaluated owing to the study design. A prospective study will be helpful to determine the true prevalence of gender dysphoria among patients with DSD.

The risk of developing malignancy within the first two decades of life is 20-30% and 15-20% for complete and mixed gonadal dysgenesis, respectively.[63] Further studies are needed to assess the incidence/prevalence of malignancy among DSD with a final diagnosis of either complete gonadal dysgenesis or mixed gonadal dysgenesis.

Our study evaluated a large sample of patients with DSD, but with the small subgroup analyses, either a type I or type II error is possible.

Conclusion

Our data have confirmed the high proportion of OT DSD in the 46, XX DSD group in South Africa, and the reasons for this need to be determined. Due to the limited availability of genetic testing in our setting, more than half of the 46 XY DSD group had no final diagnosis made. With improved availability of genetic testing at affordable cost, the final diagnosis of the 46, XY DSD group will likely change as the reason for the high proportion of OT DSD in the 46, CC DSD group.

Significantly, our results provide evidence that testosterone concentrations at presentation do not distinguish the categories of DSD. Although concordance exists between sex assigned at birth and gender of rearing in the majority of our cohort, future research should be undertaken to identify markers predicting non-concordance and the prevalence of gender dysphoria in this group of patients.

Acknowledgement

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Statement of Ethics

The study was completed by the Declaration of Helsinki and approved by the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, reference number HREC REF: 037 /2022 (Appendix 1).

Furthermore, the hospital research committee approved the study (Appendix 2). Informed consent was not obtained from individual patients or caregivers because the data was collected retrospectively. Patient details were anonymised before data analysis.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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This study was unfunded.

Author Contributions

Ewuraa Abena Manu collected the data from the RCWMCH and GSH patient files and the NHLS results database for the investigated cases and wrote the manuscript. Ian and Ariane provided guidance on the study's title and objectives, literature review, data analysis, and manuscript development. Michelle assisted with the study protocol. All authors reviewed and approved the final draft.

Data Availability Statement

The data supporting this study's findings are not publicly available because they contain information that could compromise the privacy of research participants, but they are available from E. A. Manu at ewuraa21@gmail.com upon reasonable request.

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Figure Legends

Fig. 1. Diagram showing the concordance between sex assigned at birth and assigned gender of rearing after evaluation in patients with a 46, XY DSD.

Fig. 2. Diagram showing the concordance between sex assigned at birth and assigned gender of rearing after evaluation in patients with a 46, XX DSD.

Appendices

1.DATA COLLECTION SHEET

Attached to documents

post corrections\DATA COLLECTION TOOL APPENDIX 3.xlsx

2. FACULTY OF HEALTH SCIENCE ETHICAL LETTER

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

19 January 2022

HREC REF: 037/2022

Dr A Spitaels
Division of Endocrinology & Diabetes
Paediatrics Medicine-
Email: ariane.spitaels@uct.ac.za
Student: ewuraa21@gmail.com

Dear Dr Spitaels

PROJECT TITLE CLINICAL CHARACTERISATION AND EARLY OUTCOMES OF CHILDREN AND ADOLESCENTS WITH DISORDERS OF SEX DEVELOPMENT IN A TERTIARY CENTRE OF THE WESTERN CAPE IN SOUTH AFRICA-MPHIL-DR EWURAA MANU

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020; 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 January 2023.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Ewuraa Manu will also be involved in this study.

Please quote the HREC REF 037/2022 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

3.APPROVAL LETTER FROM HOSPITAL RESEARCH COMMITTEE

Western Cape Government
Health
FOR YOU

DR T KERBELKER
Acting Manager: Medical Services
Red Cross War Memorial Children's Hospital
Email: Tamara.Kerbelker@westerncape.gov.za
Tel: +27 21 658 5383 Fax: +27 21 658 5006/5 66

02 March 2022

Dr E Manu
Paediatric Endocrinology

Dear Dr Manu,

RESEARCH: RXH: RCC 316 / WC_202202_004

PROJECT TITLE: Clinical Characterisation and Early Outcomes of Children and Adolescents with Disorders of Sex Development in a Tertiary Centre of the Western Cape in South Africa

It is a pleasure to inform you that the hospital Research Review Committee has apprcved your application to conduct above-mentioned study at Red Cross War Memorial Children's Hospital until 30 January 2023.

Kindly note that this approval is subject to strict adherence to the HREC recommendations regarding research involving participants during COVID-19, dated 17 March 2020 (UCT HREC notice attached).

Yours sincerely,

DR T KERBELKER
ACTING MANAGER: MEDICAL SERVICES

AUTHORS INSTRUCTIONS: HORMONE RESEARCH IN PAEDIATRICS

Research Article

Manuscript Title

First Name(s) Surname[a], First Name(s) Surname[a], First Name(s) Surname[b], First Name(s) Surname[c], First Name(s) Surname[a]

[a] Department, Institute/University/Hospital, City, (State,) Country

[b] Department, Institute/University/Hospital, City, (State,) Country

[c] Department, Institute/University/Hospital, City, (State,) Country

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Corresponding Author:

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Department

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A short Abstract should summarize the main points and reflect the content of the article. It should be written in a clear and concise way and be structured using the following subheadings: Introduction, Methods, Results, and Discussion/Conclusion. Abbreviations used in the main text may be introduced and used. Use neither bibliographic references nor references to figures or tables in the Abstract.

Please refer to the Author Guidelines for more information about the maximum accepted word count of the Abstract in your chosen journal. Where no specific word count is provided, an abstract of between 200 and 400 words is permitted.

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The Introduction should provide a summary of the background to the relevant field of research and the specific problems addressed and should state the hypotheses being explored as well as the main goal(s) of the study. Conclusions or findings should not appear in the Introduction.

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The Materials and Methods section should clearly list all inclusion and exclusion criteria, methods of research, and variables evaluated and should state how outcomes were assessed. All terms should be adequately defined, and statistical information should be sufficiently detailed so that a study can be repeated. If your manuscript is a clinical trial, please provide the clinical trial number.

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The Results section should describe the most important findings of the study, analysis, or experiment. The most important results should be indicated, and relevant trends and patterns should be described.

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Published research must comply with the guidelines for human studies and should include evidence that the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. In the manuscript, authors should state that subjects have given their written informed consent and that the study protocol was approved by the institute's committee on human research. If ethics approval was not required, or if the study has been granted an exemption from requiring ethics approval, this should be detailed in the manuscript. Studies involving animals must have been approved by the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee and must follow internationally recognized guidelines such as the ARRIVE guidelines. Please address the following aspects in your Statement of Ethics.

Study approval statement: Provide name and affiliation of the committee who approved the study and the decision reference number. An example statement can be found here: "*This study protocol was reviewed and approved by [committee name and affiliation], approval number [XXX]*." If ethics approval was not required, or if the study has been granted an exemption from requiring ethics approval, this should also be stated, including the name of the ethics committee who made that decision.

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2 Chen C, Hu Z. ApoE polymorphisms and the risk of different subtypes of stroke in the Chinese population: a comprehensive meta-analysis. Cerebrovasc Dis. DOI: 10.1159/000442678.

Monographs:

3 Matthews DE, Farewell VT. Using and understanding medical statistics. 5th ed, revised. Basel: Karger; 2015.

Edited Books:

4 Cohen SR, Gardner TW. Diabetic retinopathy and diabetic macular edema. In: Nguyen QD, Rodrigues EB, Farah ME, Mieler WF, Do DV, editors. Retinal pharmacotherapeutics. Dev Ophthalmol. Basel: Karger; 2016. Vol. 55; p. 137–46.

Websites:

5 Karger Publishers [Internet]. Basel: Transforming Vesalius: The 16th-Century Scientific Revolution Brought to Life for the 21st Century [cited 2013 Feb 4]. Available from: http://www.vesaliusfabrica.com/en/new-fabrica.html.

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