OvoTesticular Disorder of Sex Development in Southern Africa

Rinus Wiersma

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COVER PHOTO:

Hellenistic statue of Hermaphroditus
Lady Lever Art Gallery, Port Sunlight Village, Wirral, England

This marble sculpture is a copy of a fresco from Herculaneum (destroyed along with Pompeii in AD 79 by volcanic pyroclastic flows from Mt. Vesuvius, and located at the site of the current commune of Ercolano), based on the Hellenistic statue of Hermaphrodite, a minor deity of bisexuality, effeminacy and fertility, according to Greek mythology.
The name Hermaphroditus (or Hermaphroditos) is derived from the names of Hermes (Mercury) and Aphrodite (Venus) who were his parents. The word 'hermaphrodite' has been derived from his name. From ancient times, he/she has been portrayed in Greco-Roman art as a female figure with male genitals.

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OvoTesticular Disorder of Sex Development in Southern Africa

OvoTesticulaire vorm van gestoorde geslachtsontwikkeling in Zuidelijk Afrika

THESIS

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Rinus Wiersma
born in Amsterdam

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FOREWORD

The question most frequently asked on the birth of a child is, "Is it a boy or a girl?"[1] Not, "Is it a healthy child? " or "How well is the child?" It is as if the gender of a child is a feature that, in the minds of people, appears as important as life itself.

This introduction to the subject of Ovotesticular Disorder of Sex Development (OT-DSD), or what used to be named 'True Hermaphroditism' needs to broach the topic of alternatives to a clear male-female gender divide and the significance of gender to people. In my introduction to the subject for my Masters of Medical Science degree, I used the following paragraphs, which I have loosely adapted, as I feel they are as relevant here today as they were then in 2000.[2]

Despite our liberal Western views at the beginning of the 21st Century and our earnest attempts to achieve sexual equality, the question of gender retains its significance. The Western style of 'gender equality' is not found in all cultures and societies, often the male status is still felt to be superior and a male birth is preferred.

Whether due to preference or curiosity, doctors have been asked to predict the gender of the unborn child since the first recorded medical literature and probably as early as the dawn of the human race itself.

It is understandable that the future parents have a desire to know the gender of the child in terms of expectations, choosing the name, clothing etc. Apart from this type of curiosity, there may of course be financial and political advantages to knowing the gender of the child, for inheritance and royalty.

Present-day technology is able to predict the child's gender very accurately. In the past, attempts have been less successful, but interesting! Such means of predicting the gender of foetuses have ranged from using both natural and supernatural powers. Astrology, numerology, interpretations of dreams, examination of entrails of sacred animals and magic formulae are only some of the many curious ways that have been employed in the attempts of prediction.[3]

Regarding 'acceptable' old medical methods of prediction, examples can be found in the Egyptian papyri, which are perhaps our oldest medical records. The Be'rol papyrus, written around 1350 BC, describes a method by which both the pregnancy and the foetal sex could be predicted. The method requires that two bags, one containing barley and the other wheat, be moistened daily with the urine of the suspected pregnant woman. If the barley were to germinate, a girl would be born, whereas if the wheat were to germinate a boy would be born. If no germination should occur than the woman was not pregnant.

It is interesting that despite the antiquity of the above test, a stimulatory effect on the germinating process by urine from a pregnant woman has been confirmed, although the accuracy in the antenatal sex prediction could not be verified. The method was repeated in 1933 under scientific conditions, on the presumption that the oestrogen in the urine would stimulate the germination of barley, but have the opposite effect on the wheat. The experiment reported a correct prediction in 80% of cases. The correlation between the content of oestrogens or gonadotropins in the maternal blood and the sex of the foetus could, however, not be found.[4]

More recent than the Egyptian test, was the belief in the association between males and right-sided bodily features. During antiquity the right side of the body was considered the stronger and more valuable side, just as males were considered the stronger, superior and more valuable gender. The human uterus was also believed to contain a right and left chamber. It seemed natural therefore, and this was taught by Hippocrates himself, that the male foetus was usually seated in the right and the female in the left side of the uterus.

Of interest is that right-handed signs and symptoms pointing to a male pregnancy can be found throughout history from China to Europe. It was believed that if there was more pain, a greater heaviness or earlier movements in the woman's right side, she was pregnant with a male child. Aetius, in the middle of the 6th century said that the breasts should be watched closely in early pregnancy. With a male foetus, the right breast would be larger, have earlier milk secretion, and the right areola would be larger and darker with a redder and more projectile nipple. The same belief was common among Hindus in the 11th Century.

The modern medical ability to predict the gender of the unborn child seems to be infallible and this facility is available to a large section of women wishing to know the gender of their children. Ultrasound can accurately predict the gender of the unborn child, and with the modern 3D machines it can in fact show the parents what the child looks like as well as take pictures for the 'family album'. Apart from merely 'showing' the gender of the foetus, modern technology is such that with *in vitro* fertilisation a foetus of the desired gender can be selected and impregnated into the mother.

Despite the amazing ability of medicine to antenatally predict the gender of most children, there are those patients in whom this remains difficult. Among these are children, where even at the time of birth it is impossible to state the gender of the child with any confidence. This ambiguity of gender may arise from an abnormal development of the genitalia in a gender normal child, or due to an uncertainty of the gender itself, which is expressed as an ambiguous genital system. The latter is the condition now called 'Disorder of Sex Development' (DSD).

DSD is in general terms one where the true gender identity of the patient is not clearly apparent by external examination alone. The terms DSD and ambiguous genitalia are frequently used synonymously, often they are the same. They are however different entities of gender expression that may co-exist. In DSD the patient's gender is in question e.g. hermaphroditism, whereas ambiguous genitalia describes the poor phenotypic expression of that gender e.g. cloacal exstrophy.

Children who then present with gender ambiguity may be male, female or an indeterminate gender. Their presenting genital features form part of a gender spectrum, but none look totally normal and will have some oddity that distinguishes them from the normal male or female.

Normal sexual development is a complex mechanism and even to-date not fully understood. DSD may be divided into the 3 main types, i.e. 46,XX-Disorder of Sex Development (XX-DSD), 46,XY-Disorder of Sex Development (XY-DSD) and OvoTesticular Disorder of Sex Development (OT-DSD)

On a world-wide scale, the commonest of these three types is XX-DSD, forming 75% of DSDs, and is seen in all populations and race groups. It is a result of an enzymatic deficiency in the cortisone / 11-deoxycorticosterone biosynthesis. This deficiency, commonly the 21 Hydroxylase deficiency, gives rise to a build-up of precursors, which leads to a phenotypic androgenization.

XY-DSD on the other hand is caused by a partial or complete failure of converting testosterone to 5α-Dihydro-testosterone, resulting in a defective masculinization of the perineal structures, seen as a phenotypic feminization.

The cause of the least common condition, OT-DSD is less clear, and there are four main hypotheses.[5] These state that there is either i) a mutation of an autosomal gene giving rise to the 'Y effect', ii) a translocation of the short arm of the Y chromosome to the X chromosome, or iii) a mosaic genotype and loss of some of the Y chromosome cell lines. Lastly it is suggested there is iv) an exchange of genes between the Y and other chromosomes leading to a 'Y effect'. Of these four hypotheses the first, creating a protein which acts like HY-Ag seems to be the favoured aetiology as this hypothesis is supported by XX males. These are males who are thought to be the result of a critical genetic defect, most likely an autosomal dominant mutation, which mimics the initiating role of the SRY gene in 46,XX subjects.[6,7]

This thesis discusses many aspects of OT-DSD that have been researched in an effort to assist our patients blighted with this condition. For although OT-DSD is not a life threatening condition, it dramatically alters the life for those patients, as gender affects what we do and how we do it.

Apart from the potential medical and sexual problems this condition brings with it, the psycho-social effects of such an anomaly are enormous.[8] This may not only affect the child, but also the remainder of the family. These are some of the aspects this thesis hopes to address.

Note: The terminology used in this thesis must be explained as this has changed over the past couple of years. The improved identification of the condition's aetiology and heightened awareness of ethical issues were found to warrant a re-examination of the nomenclature. The old terms were perceived derogatory and confusing by patients and doctors alike. Many of the chapters that make up this thesis on OT-DSD were written and published, prior to the changed nomenclature of DSD. Those chapters have been reviewed and up-dated, as such their terminology uses the 'new' nomenclature, the references however will still contain the 'old' nomenclature. The adopted name changes[9] are listed in Table 1.

Table 1. Changed nomenclature of DSD

New Terminology	Old Terminology
Disorder of Sex Development (DSD)	Intersex
46,XX Disorder of Sex Development (XX DSD)	Female Pseudohermaphroditism
46,XY Disorder of Sex Development (XY DSD)	Male Pseudohermaphroditism
Ovotesticular Disorder of Sex Development (OTDSD)	True hermaphroditism

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OVERVIEW OF THESIS

The patient with OT-DSD may present to the medical practitioner for a variety of reasons, but most likely for the investigation of the gender ambiguity which is the main feature of the DSD state. The classification of DSD has been based on the three main types[1] (see

Table 1. Classification of DSD

46,XX- Disorder of Sex Development (XX-DSD)
46,XY- Disorder of Sex Development (XY-DSD)
OvoTesticular Disorder of Sex Development (OT-DSD)

Table 1).

This thesis is about children born with a gender anomaly and specifically about those with OT-DSD. It is based on research done over a period of 23-years (1984-2006) on patients managed by the Department of Paediatric Surgery, University of KwaZulu-Natal, Durban, South Africa. The patients who were referred to our clinics came from KwaZulu-Natal, Eastern Cape, Lesotho and Swaziland, thereby constituting a Southern African group.

This document is a collection of updated retrospective articles, the originals being presentations to scientific bodies and papers published in peer review journals over the period of study[2,3,4,5,6,7,8]. The original papers presented an increasing number of patients over the years. By updating the information, the thesis presents a single cohort and allowed a re-evaluation of the aims of study.

The thesis describes the studies done on patients with OT-DSD, which is generally regarded as uncommon condition among the disorders of sex development elsewhere in the world. Locally this condition is seen commonly, the reasons for the locally high incidence remain unknown.

The topics that formed the subjects of these papers were formulated from the questions posed to me by the parents of the children with OT-DSD, and to which no answers could be found in the literature at that time. This research led to a keen interest in this fascinating subject.

The format of this thesis has divided the chapters into four parts:

Part 1. Presentation of patients with gender ambiguity
Part 2. Investigation of OT-DSD
Part 3. Management of patients with OT-DSD
Part 4. General discussion

Note: It should be noted that as each chapter was an independent presentation representing a new aspect of OT-DSD, there is a certain amount of overlap of material in each to explain the background of the research.

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Part 1

The presentation of patients with gender ambiguity

INTRODUCTION

The pronouncement of the child's gender at birth is important, and for children with a gender ambiguity, this may result in serious and far reaching problems. Although gender equality is attempted in some cultures and societies, it is found in few. In most societies the male status is still felt to be 'superior' and although career choice is becoming more equal, a male birth is preferred as is the case among the black African people.

Recognition of an ill-defined or a non-existent gender is the first step toward investigation, diagnosis and management of this medical condition, called Disorder of Sex development as already mentioned and described in the introduction and overview of this thesis.

Under ideal circumstances, gender ambiguity should be recognised in-utero or at the child's delivery.[1] The recognition of such an anomaly should then be followed by early investigations, a diagnosis of the condition, a gender assignation and the commencement of a management plan.

Enabling such a series of events to proceed expeditiously requires a process of investigation that is quick, accurate and done with a sense of urgency. All this should happen prior to the newborn child leaving the hospital, such that at the home-coming, the parents can inform the family and friends of the child's gender with some confidence and without the embarrassment of a gender change at a later date.

This first part consists of two chapters regarding the general aspects of gender ambiguity.

The first chapter looks at the challenges of gender ambiguity, the second looks at the clinical approach to diagnosing DSD.

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Chapter 1

Challenges of children born with ambiguous genitalia

ABSTRACT

The meaning of gender ambiguity and the types of Disorders of Sex Development are discussed.

This chapter examines in some detail the normal development of the foetal genitalia and complex process of gender formation. It also studies where this gender development has been altered in the formation of gender ambiguity.

The effects of gender ambiguity on the child and on the parent are examined in this chapter. The issues such as the choice of gender, puberty, the management of the child's sexuality, parenting choices, and medico-legal pitfalls are discussed.

INTRODUCTION

Most people who see a newborn child with gender ambiguity may not even recognize there is a problem. At this stage the child's prospects in life are equal to those of normal children. However, due to our cultural and social norms some of these prospects are altered for children with genital ambiguity. The challenge in caring for such children is to provide them with equal opportunities in life.

NORMAL DEVELOPMENT

In a discussion on gender and ambiguous genitalia, it would be proper to discuss this in relation to the normal male and female gender developmental.

Humans have 46 chromosomes in each cell of their body, or 23 chromosomal pairs. It is the 23rd pair that determines our gender. Females have two X chromosomes, while males have one X and one Y chromosome. The chromosomal complement in humans, called the Karyotype, is written as 46,XX for females and 46,XY for males.

There is considerable evidence to show that the rate of development of the two genders is different, with the XY bearing embryo developing a little faster than that of XX embryo from the very beginning.[1] It is also noted that the right side of the foetus develops sooner than the left**.** The difference in growth rate is accentuated in the developing gonads from the gonadal ridge formation onwards. The cause for this growth differential remains unknown, but continues throughout pregnancy, with the average weight of boys being 100 gm more than that of girls at the time of birth.[2]

The direction of normal sexual development primarily depends on the chromosomal make-up, but other factors appear to play a part. The normal development of the foetus, through the action of the WNT-4 gene which is geared for ovarian production, is into a female child unless there is intervention of a Y chromosome axis. In the presence of the testicular stimulating factor (HY-Ag) provided by the Y chromosome, sex reversal occurs**.**[3]

Prior to the 7th week of gestation, the two genders are indistinguishable as they both have a rudimentary Müllerian and Wolffian system, as well as an indifferent gonad with the potential to become a normal ovary or testis.

The indifferent gonad has developed from the gonadal ridges under the guidance of the SOX-9 and DMRT 1,2 genes. The ridges themselves are formed due to the action of WT-1 & Sf-1 genes on the intermediate mesoderm**.**[4]

The Y-chromosome has only one function to play in the determination of gender, and that appears to be in the development of testicular tissue.[1] On the short-arm of the Y-chromosome, situated in a peri-centromeric position, is the SRY-gene (sex-determining region of Y-chromosome) consisting of 79 base pairs, and is responsible for the production of Testis Determining Factor, also known as Histocompatability-Y-Antigen (HY-Ag), which is a DNA binding protein.[5] The action of the HY-Ag provided by the Y chromosome, switches the WNT-4 gene off and causes a reversal of the gender.[3]

The change from indifferent gonad to testis is dependent on several factors. The gonad needs to have reached a specific size at a given stage of embryogenesis for it to be sensitive to SRY. The SRY then switches the WNT-4 gene off in the time interval 7-9 weeks gestation, which leads to testicular development. Failure of any of these factors results in the indifferent gonad developing into a normal ovary.[6]

The male development commences with the development of two cell lines, both of which go on to produce hormones that direct aspects of male differentiation. The first of these cells to develop are the Sertoli cells. These organize the surrounding cells into interconnecting tubular structures called the seminiferous cords and produce AntiMullerian hormone (AMH, previously known as Mullerian Inhibiting Substance). Its action causes the regression of Müllerian ducts in males, but only during a "window period" from the 6^{th} to the 9^{th} week gestation.[7] Before or after this period AMH has no effect on the Müllerian ducts. The absence of AMH results in the Müllerian ductular system developing into fallopian tubes, uterus and upper 1/3 of the vagina. In males, serum AMH values remain high (10-70 ng/ml) for several years after birth, then decline to a low adult level (1-5 ng/ml) at puberty.[8] In contrast, AMH is undetectable in the normal female before the onset of puberty, at which time it becomes measurable in the serum at 1-5 ng/ml. In females AMH is secreted by the granulosa cells of the ovary and it is postulated that it plays a role in the regulation of oocyte maturation.[9]

The second line of cells are the Leydig cells. These cells take up C21 steroids of placental and foetal adrenal gland origin and produce testosterone.[10] This hormone now becomes responsible for the further development of the male genitalia.[2] The origin of these cells is still unclear, one suspects that a steroid-producing population of cells in the ventral part of the mesonephros differentiates to form both the origins for the Leydig cells and the adrenal cortical cells.[11,12.]

Testosterone is required for male sexual development;

- Firstly in the structural alteration of the brain of the foetus.[13,14] It has been shown that endogenous hormones influence gender differences of the brain. The neonatal testosterone produced by the foetus takes the main role in the irreversible masculinization of the brain.[15]
- Secondly the testosterone is altered by the perineal tissues with androgen target cells containing the enzyme 5-α-reductase to become 5α-Dihydro-testosterone (DHT). DHT combines with a cytosol androgen receptor and stimulates tubularization and advancement of the urethra and enlargement of the foetal phallus to become a

penis. The scrotal folds then fuse in the midline. Testosterone also allows the Wolffian structures of prostate and the ductular system to grow.[16]

The long-arm of the Y-chromosome provides factors that allow maturation of spermatogonia and in turn produce spermatozoa. The remaining portion of the long arm of the Y-chromosome appears genetically inactive, as far as can be determined at this stage.[10]

Gonadal differentiation in the female, lags behind that of the male counterpart, having waited for the HY-Ag stimulus. Once the window period is over, the WNT4 gene action makes the indifferent gonad sensitive to circulating maternal oestrogens. The oestrogens stimulate the development of the follicles, which then produce their own oestrogen. The foetal oestrogen allow the Müllerian structures to grow and fuse in the pelvis to become the uterus and upper vagina. The medullary tissues regress, resulting in the absence of testosterone production and in turn to the regression of the Wolffian ductular system.

There have been proposals that the female embryo has it's own stimulus to develop an ovary and that this can be attributed to the XX chromosome.[17,18] This has been named the 'Meiosis Induction Factor', which prevents the action of HY-Antigen. Although this agent has been demonstrated in mice and has been proposed to be the cause of ovotesticular tissue in OvoTesticular-DSD (OT-DSD) subjects, this has as yet not been isolated in humans.

The agonadal foetus, due to the lack of testosterone, develops into the phenotypic female, regardless of the genetic make-up. There is some evidence that the ovary is not the driving force in the sexual differentiation of the female, as the testis is in the male.

As a result of these two processes the foetus with a 46,XY karyotype will develop into a normal male and with a 46,XX karyotype will develop into a normal female. To date, however, no specific gene mutations can be demonstrated in a majority of patients with sexual ambiguities or reduced fertility.

Regarding patients with OT-DSD, local studies have shown some features that may have a genetic basis. These being that the condition is seen most commonly among the black African population, whilst rare among the other race groups, and familial cases are rare in these patients.[19]

The genetic make-up of OT-DSD patients is not homogeneous. The karyotype in the majority of South Africa OT-DSD patients was found to be 46,XX, in Europe there appears to be a high preponderance of Mosaic 46,XX/46,XY carriers and in Japan the 46,XY karyotype predominates.[17,18]

The investigation of patients with OT-DSD has thus far shown that there is no clear designated cause for this condition. A review of 283 OT-DSD patients in the literature looked at the information available on the causes of OT-DSD, but could not define any other than the original four postulated causes, as outlined in the foreword to the thesis.[20] One of the assumptions is that there are multiple H-Y structural genes on the Y chromosome and that if these are split by translocation to another chromosome, the number of gene copies translocated may determine the dosage of testicular development in the gonadal differentiation.[7] The development of the ovotestis then leads to the secondary changes in genitalia of the patient. With genetic analysis becoming more readily available, this is where future investigation of OT-DSD should be directed.

WHAT IS GENDER AMBIGUITY?

Gender ambiguity is a condition where the true gender identity of the person may not be obvious by looking at the external gender appearance alone.

WHAT CAUSES AMBIGUOUS GENITALIA?

The causes of gender ambiguity fall in two main groups.

- In the first group are the patients who have a normal gender, but from their phenotype, i.e. genital development, it is difficult to discern what that gender is. Structural malformations such as hypospadias and bladder exstrophy etc, fall into this category.
- The second group are those patients with Disorders of Sex Development (DSD). Here due to chromosomal, hormonal or enzymatic influences there is a duality of gender expression, resulting in a mixed gender phenotype.
  DSD conditions have traditionally have been classified as three main types, although newer classifications have recently emerged and add a fourth type:[20,21]
- 46,XX-Disorders of Sex Development (XX-DSD). – World-wide, the commonest cause is Congenital Adrenal Hyperplasia. These are genotypic females.
- 46,XY-Disorders of Sex Development (XY-DSD). – The commonest cause is due to testosterone insensitivity. These are genotypic males.
- Ovotesticular Disorders of Sex Development (OT-DSD). – The cause remains unknown. Patients may be genotypically female, male or rarely have a moziac or chimeric karyotype.
- 46,XY Complete Gonadal Dysgenesis.

EFFECTS ON THE CHILD

Firstly the effects on the child may be that of being different in looks and not like their peers, particularly at the time of noticing gender differences (3-4 years of age) and later during dating. The vast majority of such patients are sterile and will not be able to parent offspring.

What gender is the child to be brought up in (Male / Female/ Indifferent?)

This depends on the underlying gender of this child.[22] There is of course only the choice between male and female, although there had been a move among interest groups in the developed world to raise these people in a neuter gender. In the children with XX-DSD and in testosterone insensitivity the underlying behavioural gender is female. In the OT-DSD there is no underlying gender, the medical staff need to wait to see what gender behaviour is displayed at 6-8 years of age.

Who makes the child's future gender decision?

Ideally this decision should be made by a team of specialists who have assessed the underlying cause of the problem, and the likely gender the child will develop.[23] The findings are discussed with the parent and the child, if old enough to understand, before the most appropriate gender for raising the child is suggested.

When can the child make the decision regarding its own gender?

Gender identity emerges at 2-3 years of age, and by the third year most normal children can identify adults as males and females. By age 6 years most children identify gender and spend more time with their own gender than with the opposite gender. Children develop gender- identity constance between 5-6 years of age.[24] Children with DSD however show some confusion, but should be capable of assessing its own gender at about 6-8 years of age. It is this ability that will assist the medical staff in the gender choice thereafter. It is wise to heed that gender choice from then on, but the child is a minor up to 18 years of age and legally only gets a real say in the decision after that age.

What functional and cosmetic changes need to be brought about?

Once the gender choice has been made, a decision is needed regarding what to do with the gonads? Should the gonads be left in situ if they are in keeping with the patient's gender choice, or be removed?

When is it an appropriate time for cosmetic surgery?

In some DSD patients where the gender for raising the child has been determined early, the condition will allow and indeed is best managed with early 'cosmetic surgery' e.g. among the XY-DSD with testosterone insensitivity.[25] In the XX-DSD some time should be allowed to ensure that medication to correct the biochemical defect and stop further androgenization is established before clitoral reduction surgery is commenced.[26,27]

Children with OT-DSD on the other hand require a wait and see policy, to evaluate what gender the child thinks it should be before corrective surgery is attempted.

The management of puberty

Where there is no or insufficient gonadal tissue to induce normal puberty, artificial hormonal induction is a necessary step. The age of inducing puberty is dependent on the patient's height and psycho-social factors, such as the stature with respect to family, hair growth and mental maturity.[20]

Male puberty is induced in such children using testosterone, with increasing dosage over a two year period to reach an adult dosage. In females, puberty is induced with ethinyl estrodiol, again giving an increasing dosage over a two year period. In the latter stages, progesterone is added daily and then cyclically to produce menses.[20] During the period of inducing puberty, the growth plates stop growing and the diaphysis and metaphysis fuse, usually adding another 20 cm (females) to 25 cm (males) to the total height from start to completion. Once the growth has stopped and secondary sex characteristics have developed, the treatment needs to be continued life long in those children who have no gonadal function.

Sexual intercourse & Fertility

The commonest form of DSD in the world, XX-DSD is the only form where fertility is said to be unaffected by the condition. It is certainly the aim of the medical fraternity to restore normal sexuality and fertility to all where that is possible. Despite this, patients with XX-DSD have difficulty in fitting into the normal 2 gender society, 50% abstain from sex, 25% are lesbians and the remainder are said to have 'normal' sex lives.[28,29]

EFFECTS ON THE PARENTS

For the parents of children with ambiguous genitalia, the stigma of having a "different" child is equally worrying and may be embarrassing. The majority of parents are highly sensitive about their children's anomaly and hide the fact from even the closest of family and friends.[14,29,30]

Can the parents decide on the gender for the child (Is this parenting?)

In cases where the medical problem is difficult, it would be irresponsible to leave the decision of gender solely to the parent, even if they have already made a choice. Primarily because parents often may have no appreciation of the medical problem and secondly as in the case of the child with XX-DSD, there is already a definite underlying gender.

Underlying problem? (Hereditary/ Isolate problem?)

Although children with XX-DSD are capable of bearing offspring, and isolated examples of fertility are noted among patients with OT-DSD, all the other types of DSD patient tend to be sterile. It is therefore unlikely that this condition is going to be passed on to the next generation, or that this was inherited from either parent.

Cultural / Social forces

Social forces are brought on parents to decide early what gender the child is. That is in terms of choosing a name for the child, the colour of the clothing, gender based play schools, which toilets to choose etc.

Apart from the potential medical and sexual problems this condition brings with it, the psycho-social effects of such an anomaly are enormous.[28,29] Firstly the effects on the child may be that of being different in looks and not like their peers, particularly at the time of noticing gender differences (3-4 years of age) and later dating. As the vast majority of such patients are sterile they will not be able to parent offspring, which is the cause of considerable grief in the family.

LONG-TERM OUTLOOK FOR CHILDREN WITH AMBIGUOUS GENITALIA

Making a correct determination of gender is important for both treatment purposes, and the emotional well-being of the child and family. Some children born with ambiguous genitalia may have normal internal reproductive organs that potentially should allow them to live normal, fertile lives. However, the majority may experience difficulty with their chosen gender, their sexuality and their lack of fertility.[31]

MEDICAL/LEGAL PITFALLS

The ideal management of the newborn with ambiguity of the genitalia is a team approach including neonatologists, geneticists, endocrinologists, surgeons, counsellors, and ethicists. Adequate counselling and support for parents is vital. From a medico-legal standpoint, the best approach to managing these patients is to provide parents with as much information as possible so that they can make informed decisions. Despite this, changing one's gender socially and in the national registry can be embarrassing and a total administrative nightmare, even for those with normal gender development.

The treatment for patients with DSD is controversial.[26] No one debates the need to address and treat underlying physiological problems such as those associated with XX-DSD. The controversy revolves around issues of gender reassignment. Gender assignment by the physician and family may not correlate with gender preference of the patient in adulthood, bearing in mind that the most important sex orientation organ is the brain, which has already undergone hormonal imprinting in-utero.

Activists for patients with DSD and some health care professionals have called for a moratorium on gender reassignment and genital surgery until studies have been completed on the long-term effects of such surgery. Several long-term follow-up studies are being conducted, including a study by the North American Task Force on Intersexuality. Many health care professionals oppose the proposed moratorium.[23]

Once the gender for the child has been chosen, this should now be 'formalised' and noted by the authorities. To change this gender through the legal process is a lengthy legal nightmare, especially for those sensitive to the issue. On top of this is social status of the person. To change one's gender socially has a serious stigma attached to it. Therefore a wise initial choice is an important issue.

CONCLUSION

Examination of the newborn child is important both in terms of timing and assessment of the normality of the organ systems. The effects of a misdiagnosis of the genital system are as serious as they are for any other organ system in the body, but are not lethal.

The psycho-social impact of the genital anomaly is enormous on both the child and the parents. These effects are life-long, where the majority of patients may experience difficulty with their chosen gender, their sexuality and their lack of fertility. The gender choice should therefore be made with these consequences in mind.

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Chapter 2

A clinical approach to diagnosing Disorder of Sex Development

This Chapter is based on Wiersma R. A Clinical approach to diagnosing Intersex.

***South African Paediatric Review*, 2004; 1(3):15-23.**

ABSTRACT

Recognition of patients with Disorder of Sex Development and the understanding of the causes of this condition occurred during the 1980's, at the time this research commenced.

This chapter discusses the clinical features to look for in patients with ambiguous genitalia. The biochemical, hormonal, chromosomal as well as the radiological investigations of ultrasound and genitograms are discussed and their diagnostic value are noted.

Locally, a diagnosis can be made by chromosomal assessment, clinical and endoscopic evaluations together with gonadal biopsies. These features are discussed.

INTRODUCTION

Available records of the Department of Paediatric Surgery, University of KwaZulu-Natal, Durban, South Africa show that patients with ambiguous genitalia have been recognised and treated in the Durban Metropolitan Hospitals since the early 1960's.

It was only as recent as the 1970's that parents were advised to bring children with ambiguous genitalia back to hospital after 18 months, as surgery at that time was 'not done on such young children.[1] The result was that of the 25 children with ambiguous genitalia during the years 1970-1979, only 4 were fully investigated and given a diagnosis.[1] The remaining 21 children went home after their first superficial examination, without a diagnosis, definitive treatment or a follow-up appointment. Even in the late 1980's establishing the true gender of these children with Disorder of Sex Development (DSD) was still a haphazard and time consuming procedure.

The literature on the subject of investigating such children was sparse although the problems of DSD and its many ramifications were well recognized.[2,3] Despite the fact that more literature is available today, none address the problems faced locally in the Third World setting.[4,5]

DEFINITIONS

Although the terms 'ambiguous genitalia' and 'DSD' are liberally used, the accepted definitions of these terms are important.

Ambiguous genitalia is where the gender of the child is not clear at first inspection. This may be due to structural anomalies e.g. bladder exstrophy.

Disorder of Sex Development is a condition where the patient has both male and female genital characteristics. This may be due to any number of causes, the commonest on a worldwide scale would be 46,XX-DSD.

The '**phallus'** is defined as the common structure seen in both the male and female foetus. This structure, as a result of androgen stimulation, enlarges to form the **'penis'.** Without such androgen stimulation, the phallus remains rudimentary, constituting a **'clitoris'.** In this thesis the distinctive sizes used for the clitoris is <1.0cm in stretch length, a small penis is between 1.0-2.5cm stretch length, a normal penis is 2.5-3.5cm stretch length and a large penis is >3.5cm stretch length.[6,7]

The differences between **'labia'** and **'hemiscrotal folds'** are that the scrotum has rugae and is prouder due to distension by genital structures, i.e. a gubernaculum or gonad.

AETIOLOGY OF DSD

A practical classification based on the aetiological causes is provided here, although newer classifications are suggested in the literature.[8,9]

- o **Abnormal chromosomal development**. Examples are whole chromosome anomalies (e.g. *Klinefelters, Turner*), or gene mutations of the SRY gene, giving rise to XY females with gonadal dysgenesis (e.g. *Swyer syndrome*); or translocation of part of the Y chromosome containing this gene to the X chromosome causes *XX male syndrome*.
- o Normal chromosomes, but **abnormal gonadal development** (e.*g. Gonadal dysgenesis, Ovotesticular Disorder of Sex Development*).
- o Normal chromosomes and gonadal development, but **abnormal genital development due to a biochemical lesion** (e.g. *46,XX-Disorder of Sex Development*).
- o Normal chromosomes, gonads and biochemistry, but **abnormal genitalia due to an end- organ insensitivity** (e.g. *Androgen insensitivity syndrome, 46,XY-Disorder of Sex Development*).
- o Normal genital precursor pathway, but **abnormal genital development on the basis of a congenital development** (e.g. *severe hypospadias*).

The modern classification and terminology of patients with ambiguous genitalia has been based on the three main manifestations of gender ambiguity which are 46,XX-DSD, 46,XY-DSD and OvoTesticular- DSD.[10]

INCIDENCE

The incidence of DSD is generally the same the world over. 46,XX-DSD is the commonest cause of all, with OT-DSD being generally regarded as an uncommon cause, comprising 3-10% of all causes.[11] In Southern Africa this condition has been found to have a peculiar high incidence of 51% of the DSD patients studied.[12]

ASSESSMENT OF THE CHILD

A functional assessment of the patient's gender status and a possible diagnosis of the condition can only follow a full evaluation of the patient. Patients who were seen and managed in the Department of Paediatric Surgery, University of KwaZulu-Natal, Durban South Africa for DSD had both a *clinical* and *investigative* assessment.[13]

Clinical assessment

It is often the initial clinical evaluation of the patient that suggests that the gender of the child is in question. The majority of such patients have an obvious external genital ambiguity, and the appearance may form a full genital spectrum from male to female. This spectrum is represented in the Prader classification of genital appearance.[14] Some patients may appear to be 'normal' males or females at first glance, and have sufficiently subtle signs to delay detection. Such patients may not seek medical advice and are only diagnosed at routine medical examination or ultimately at autopsy.[11,15]

With the general examination, one must look for any feature of genital abnormality, which may suggest the presence of a DSD condition, e.g. cryptorchidism, hypospadias, or an increased pigmentation of the areola and labioscrotal fold. DSD states may be associated with certain syndromes, which might be the reason for investigating the true gender of the child, e.g. the webbing of the neck and short stature in the child with XO-karyotype Turners Syndrome.

In the older child, the parent may find the child's behaviour inappropriate for the assigned gender. This is obviously important in the psychological management and subsequent gender in which the child is raised.

Ideally the first investigations should be done before the 2nd birthday, as a change in gender assignment after the 2nd year of age is associated with severe psychological problems in the child and family.[16] Gender identity will be established from the 5th year of age and most children will know what gender they identify with by 8-10th years of age.[17,18]
Specific clinical features to look for in external genitalia are the following:

Breast development

In older children with functional ovaries, the stimulation of the breast tissue may occur around the time of puberty, but precocious or delayed development may be a feature of abnormal gender development. It should be noted that breast development may also be seen in those children who have mistakenly ingested the mother's oestrogen containing pills as 'sweets'.

Development of the penis

Clinically the majority of children with DSD show some effects of virilization. This is on the basis of either testosterone dominance or the presence of other androgenic hormones. In females with 46,XX-DSD, the clitoris enlarges due to the andrenogenic properties of the cortisol precursors. In patients with OT-DSD, despite the presence of both functional ovarian and testicular tissue during the foetal period, there is the tendency to develop a penis due to the masculinizing effects of the testicular hormones. Although some of our patients had a clitoris, the majority were male-like structures of an appropriate for age size, but here too there was a range in infants from small (<2.5 cm in length) to large (>3.5 cm in length).[6,7]

Labio-scrotal folds

The 'labio-scrotal' structures are often seen as indistinct bifid folds. If these labio-scrotal folds are flat and smooth they look like 'labia', but if they contain a tubular or gonadal structure, they would develop rugae and are called 'scrotal' folds. Scrotal folds that are fused would appear as the normal scrotum, which generally moves the urogenital orifice ventrally.

Among the patients with 46,XX-DSD and 46,XY-DSD the majority of patients had labial folds. The majority (76%) of OT-DSD patients either had bifid labio-scrotal folds or a combination of a labium with a hemiscrotal fold. Only 7% of patients had normal female labia and 17% had a normal male scrotum.[15]

Perineal orifices

The perineal orifices most commonly seen are a normally placed anus and a ventral 'urogenital sinus', i.e. a common terminal opening for both the urethra and a Müllerian structure. Occasionally these orifices are noted as a separate perineal urethra and vagina, but this is reported in only a minority of such patients.[13,17] Those children with a separate perineal urethral opening, are rarely found to have the urethra open on the penile glans.

Gonads

Gonads in patients with ambiguous genitalia may be situated anywhere in a line between the kidney and the labioscrotal fold. If the gonad contains testicular tissue, it is usually found in the labio-scrotal fold, and the descent was usually seen unilaterally.

The palpable gonad usually contains testicular tissue, although in 26% of patients the labioscrotal fold may contain ovarian tissue.[20] In the case of OT-DSD where there is testicular and ovary tissue, if the gonad contains testicular tissue, it is more commonly felt on the right (59%) than on the left side (41%).[21]

The absence of bilaterally palpable gonads renders a patient with cryptorchidism and hypospadias indistinguishable from the DSD patient. Local knowledge of the incidence of DSD would be important in the investigations of such patients.

Several articles have stated that it was possible to distinguish between testicular and ovarian tissue on palpation of the external gonad. Testicular tissue was found to be soft on palpation, whereas the ovary was a firmer structure.[20]

Investigations

These investigations are done to find the aetiology of DSD and assist with management of these patients. The following formed the bulk of the investigations.[13]

Serum urea and electrolytes

These are done with a sense of urgency to exclude a salt losing adrenogenital syndrome the commonest cause of DSD worldwide. Locally few such patients were seen. All other DSD types have normal biochemistry.

Steroid assays

This series of tests looks at the various androgenic and oestrogenic hormones and their precursors, of note being cortisol, testosterone, 17-OH-Progesterone, ACTH and DOC. Although these steroid assays are direct measurements of the patient's blood values, the 'testosterone stimulation test' is an indirect assessment of the patient's testicular hormone production, giving a measure of functional testicular tissue. This is done by giving the patient a testosterone precursor ß-HCG for three days and measuring the responding level of testosterone production.

The general pattern of steroid chromatographic assays shows children with 46,XX-Disorder of Sex Development (XX-DSD) to have a low cortisol, elevated ACTH and a relatively low 11-deoxy-cortisol. These features are in keeping with the biochemical findings of **XX-DSD** with a 21-hydroxylase deficiency. Figure 1 schematically shows the steroid pathways and important enzymes as numbers, i.e. '21' for 21-Hydroxylase enzyme deficiency, which leads to an accumulation of androgenic precursors. Other biochemical support for a diagnosis of congenital adrenal hyperplasia is an elevated testosterone (the product of an enzyme deficiency) and progesterone level. A common differential diagnosis for 21-hydroxylase deficiency is 11-hydroxylase deficiency. A relatively low 11-deoxy-cortisol suggests that this is an uncommon cause of congenital

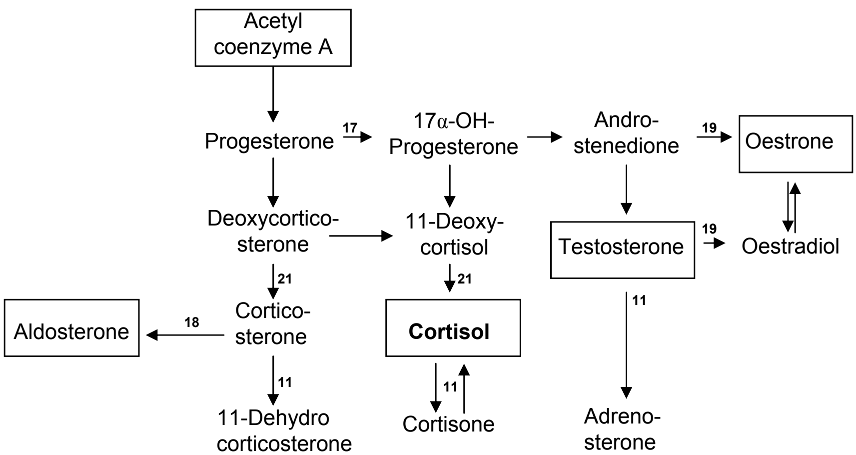

Figure 1. Schematic representation of the Enzymatic Steroid pathway

adrenal hyperplasia, a finding consistent with the pattern of enzyme deficiencies seen in other countries.[10]

A relatively low random testosterone and low ß-Human Chorionic Gonadotropin response was indicative of a functional testicular tissue deficiency. Children with mixed gonadal dysgenesis and hypospadias had an essentially normal hormone profile.

The steroid profile of patients can therefore be used to draw a distinction between female pseudohermaphroditism and the other DSD forms, but no other deductions can be drawn from the steroid assays as the remainder of the DSD types have a nondescript steroid pattern.

Chromosomal studies

This investigation is done looking for chromosomal anomalies e.g. Turners, Klinefelters, mozaic or chimeric variations of the normal karyotype. Although this investigation is now done routinely, this was not the case in the early days of investigating such patients.

Chromosomal anomalies may be seen among the patients with DSD, although the most common DSD, i.e. patients with 46,XX-Disorder of Sex Development, have a normal female karyotype. Mosaic and Chimeric chromosomal patterns are unusual among the patients with DSD.[6,21]

The chromosomal patterns of patients with OT-DSD studied locally showed that 85% were found to have a normal 46,XX karyotoype, seven patients had a 46,XY karyotype and only three patients had a Mozaic/ Chimeric karyotpye. Sex determining locus-Y (SRY-gene) translocations were looked for in recent years, but not found in any of the patients. Such translocations have often been speculated to be the cause of OT-DSD. This has not been substantiated on chromosomal assay, probably due the fact that up to that time such portions of chromosomal matter were too small for detection.[22] Elsewhere in the world OT-DSD patients are found to have different chromosomal patterns. In Japan the majority of patients have a 46,XY chromosomal pattern, whereas in Europe an even distribution of 46,XX and 46,XY patients are seen.[19,23]

Ultrasound & Genitogram

Ultrasound investigations may show the presence of gonads and Müllerian structures. The genitogram is where a fine catheter is introduced into the urogenital sinus up to the bladder neck. Under screening X-ray, contrast is introduced as the tube is slowly withdrawn, looking for the opening of a Müllerian structure.[24]

In the small child with ambiguous genitalia, these two investigations should locally be replaced with a urethroscopy and laparoscopy, due to the high incidence of OT-DSD and need for gonadal histology, whilst there is a local lack of radiographic expertise.

Urethroscopy

This procedure, inspecting the urethra for normality, is done as part of the examination under anaesthesia in theatre. The position of the urethral opening is noted, together with the presence or absence of a prostatic urethra with a verumontanum. The dorsal surface of the urethra, between the perineum and bladder neck, is carefully inspected for an opening of the vagina or some remnant of the Müllerian system.

The presence of Müllerian structures opening on the urethra may be seen in any of the DSD conditions. This investigation is therefore vital for the further management of the child. Not all children with a uterus have a vagina and vice versa.

Laparoscopy

Laparoscopic examination for DSD should be done through an umbilical endoscopic-port, preceded by emptying the bladder and stomach. The position, gross appearance of the gonads and Müllerian &/or Wolffian structures present are noted. This procedure may be performed safely in neonates as young as two-days old.

Gonadal biopsies

Following inspection of the internal genitalia with the laparoscope, biopsies of the gonads may be done via a second and third ports inserted bilaterally. Here the port size of 5mm or larger is important as this should allow the infant gonad through without damage. Where the gonad appears abnormal, the gonad is delivered through the port and a pole-to-pole wedge biopsy is taken and sent for histology.

As the diagnosis of OT-DSD is based on gonadal histology and this condition has a high incidence locally, it is advisable to do gonadal biopsies in children investigated for ambiguous genitalia with abnormal looking gonads.

IMPORTANCE OF THESE INVESTIGATIONS

The investigations of patients with DSD are directed toward defining the underlying condition. The normal investigations for DSD, e.g. assessment of salt loosing states, chromosomal make-up and steroid chromatograms, should be done to identify any of the more frequently seen causes of DSD. However, the analysis of investigations on our DSD patients found that only three investigations are initially required to make a diagnosis. These are the chromosomal assay, the urethroscopy and the gonadal biopsy. These three investigations will yield the following:

Chromosomal studies help to identify female, male and mosaic karyotype patterns. Although the chromosomal results have little bearing on the ultimate gender of rearing, it is a useful investigation as it gives the genetic gender of the child. Where a 46,XX karyotype may identify the patient with 46,XX-DSD or OT-DSD. The 46,XY karyotype indicates the patient with 46,XY-DSD, mixed gonadal dysgenesis, OT-DSD or hypospadias / undescended testes in the male child. The mosaic patterns appear in patients with mixed gonadal dysgenesis or OT-DSD.[21]

Examination of the external genitalia in small children is difficult if there is some ambiguity. This is more easily and completely done under sedation or under general anaesthesia if part of another procedure. The perineal genitalia as well as secondary sexual features such as pubic hair, axillary hair and breast enlargement are examined.

Fiberoptic inspection of the urethra is a quick and accurate method of assessing the presence of Müllerian structures. This investigation helps with the diagnosis of DSD condition and allows the investigator to obtain an accurate assessment of the size of any vaginal structure and relative difficulty in exteriorising such a structure in later years (>12 years). Laparoscopic inspection of the pelvis provides a clear view of the internal genitalia and position and structure of the gonads.

Gonadal histology allows a clear distinction to be made between the patients with the various DSD types. This investigation provides the correct diagnosis of the underlying DSD problem and the information required for making management decisions. Further specific investigations may be necessary for metabolic or hormonal studies.

The significance is that the above investigations can be done in most hospitals and by most surgeons with the correct instruments. They will give reliable results and serve as a basis for further specific investigations, e.g. hormonal studies to assess the type of adrenogenital syndrome, to help plan the further management of the patient.

Analysis of our findings has highlighted several other features of the patients with DSD. First our patients showed no association between their external appearance and the diagnosis. This meant that any degree of ambiguity of the genital structures should be investigated at the earliest opportunity to make a diagnosis of the underlying condition and allow the parents to make the adjustment of gender orientation before an irreversible assignment has been made. Secondly this study also shaped the investigative methods to be done on children with ambiguous genitalia in order to provide the investigator with clear management guidelines. Lastly, once the diagnosis was made using this investigative method, further management of the patient was now dependent on other features of the patients.

The importance of gonadal potential in such a patient lies in the retention of a gonad with the hormones producing ability, which is in keeping with the gender the child is raised in. The production of the gender specific hormones means that the child would not have to take hormonal supplementation to induce puberty. The development of malignancy of gonads of patients with genital ambiguity are unusual. Gonadoblastomas and dysgerminomas have, however, been described, particularly in patients who had an XY chromosomal structure.[25,26] Embryonal carcinoma of testicular tissue has also been reported.[20]

As far as fertility of these patients is concerned, only female patients with 46,XX-DSD and males with severe hypospadias have any chance of fertility. Patients with these DSD conditions therefore need to be recognised and treated appropriately to establish their true underlying gender. 46,XX-DSD patients with congenital adrenal hyperplasia require life-long correction of their metabolic defect to render them metabolically normal. The males with hypospadias and undescended testes have reduced sperm counts, but the possibility of fertility remains. This needs to be optimized by orchidopexy at the appropriate time and maleness needs to be established. Examples of other types of DSD states bearing children exist, but the incidence is extremely low.[10] Patients with OT-DSD raised as females have been described to fall pregnant, whilst those patients raised as males have been found to produce small sperm counts, but only one case of male fertility has been described. Turner's Syndrome patients have rarely been described to fall pregnant.[10]

The family's or patient's wishes are an essential part of the management and it is vital to include the family in all the decision making, it is after all they who are going to raise the child. The parents may, however, have preconceived ideas about the gender of the child, due to social or religious beliefs. Their strong feelings for a particular gender may be contrary to what the true or most suitable gender may be for the child. However, wishes and feasibility need to be combined, which is largely based on the genital structures available and gender the child identifies with.

The ease with which the genitalia can be reconstructed is based on the anatomic structures, which are often the ultimate deciding factor in making the gender assignment, regardless of what other factors may dictate. An inadequate phallus or the lack of a vagina are real surgical management problems. These can be overcome with modern-day plastic surgery, making the child into a male if there is only clitoral enlargement without any vaginal structures, or making a female out of a child with adequate vaginal tissue ensures that best use is made of the available tissue and the child will require only minimal additional surgery to complete the gender profile.

The further management of these patients varies considerably, from primary total gonadectomy and extensive reconstruction to a more conservative type of management. All require some psycho-social help with their gender identity.[17]

Following a full discussion with the child (where possible), the parents, and those concerned with management of the patient, a change in gender orientation may be necessary.

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Part 2

The investigation of OvoTesticular Disorder of Sex Development

INTRODUCTION

The five chapters that make up this second part all examine specific aspects of OvoTesticular Disorder of Sex Development (OT-DSD).

Establishing whether a patient with ambiguous genitalia has a Disorder of Sex Development (DSD) condition, requires urgent and relevant investigations. Once a diagnosis had been made, further tests could be done to determine the specifics of that condition. The aim of the third chapter is to re-evaluate the incidence of OT-DSD since the original analysis in 1992. With a larger cohort the incidence of this condition would be more clearly defined.

Following on from the findings that OT-DSD was seen more common locally than elsewhere, our patients were studied to assess what made them different. The fourth chapter looks at the clinical presentation of OT-DSD patients with the aim of facilitating early recognition of that condition.

The single commonest gonad seen in the patient with OT-DSD is the ovotestis, which combines these two gender opposite tissues in a single gonad. The Southern African ovotesticular histology was found to be different from the description of these gonads in the literature. The aim of the fifth chapter is to describe the gonadal tissue seen in Southern African patients with OT-DSD.

OT-DSD is a condition where the gonads of that person contain ovarian tissue with follicles and testicular tissue with seminiferous tubules. These findings can only be made following biopsies from both gonads. The aim of this tissue biopsy is to obtain a representative histological evaluation for diagnostic purposes and management decisions. The sixth chapter compares the histology of gonadal biopsies with that of the subsequently excised whole gonads, with the aim of determining the representivity of the biopsy samples.

Following on from the third chapter, which showed that a complete genital examination in conjunction with gonadal biopsies was the most expeditious method to determine the cause of DSD in our environment. The seventh chapter explores this further with a comparison between the laparoscopic and open laparotomy methods of examination and gonadal biopsy. At the time that the laparoscopic method was introduced to evaluate small children with ambiguous genitalia, this was a new approach.

Chapter 3

Disorder of Sex Development: an investigative problem

ABSTRACT

This study looked at the investigative process of patients who were referred for ambiguity of the genitalia. Two-hundred and sixty seven patients with ambiguous genitalia were seen at the University of KwaZulu-Natal, Durban, South Africa, over a 23 year period (1984-2006). These were children referred from peripheral hospitals who came with an assortment of investigations.

Investigations of patient were not done according to any standardized protocol. Once a diagnosis was obtained, the management was fully discussed with parents and the combined investigations were evaluated.

In total there were 66 patients with XX-Disorder of Sex Development, 111 with OvoTesticular Disorder of Sex Development (OT-DSD), 14 patients with XY-Disorder of Sex Development, 10 patients with Mixed Gonad Dysgenesis and 17 patients who had syndromic features causing genital distortions and 49 undervirilized males.

OT-DSD was found to constitute 51% of all Disorder of Sex Development patients seen locally.

INTRODUCTION

The aim of this study was to analyse which investigations assist in making a diagnosis of the various Disorder of Sex Development (DSD) conditions, and establish the local incidence for Ovotesticular Disorder of Sex Development (OT-DSD).

PATIENTS AND METHODS

Patients

All patients with ambiguous genitalia referred to the Paediatric Endocrine or Paediatric Surgical units at the University of KwaZulu-Natal, Durban, South Africa, were included in this study. The data was available on 267 patients of all race groups seen over a 23 year period (1984- 2006). At presentation the patient's ages ranged from 1-day to 13-years, and 46% of the patients were given the primary female assignation by the parents prior to admission.

Methods

The patient's investigations ranged from clinical examination, to laboratory, radiological and histological evaluations, assessing the various features of DSD. During the study period, few of the 267 DSD patients required all of the available investigations to make a diagnosis. This is reflected in the tabulated results, which show incomplete numbers of this cohort.

The tables are divided into the six ultimate diagnoses of this cohort of patients, i.e. XX-DSD, OT-DSD, XY-DSD, Mixed Gonadal Dysgenesis, Syndromic patients and patients with undervirilizing UroGenital Syndrome. This division helped in evaluating the investigations for each diagnosis.

Blood analyses included biochemical, hormonal, and chromosomal assays. For the chromosomal assays, the standard method of looking at 20 fields for chromosomal abnormalities was applied. SRY antigen was not looked at in the majority of patients.

Radiological investigations consisted of a pelvic ultrasound and a genitogram. The pelvic ultrasound looked for Müllerian structures and gonads, whilst with the genitogram contrast was infused into the urethra looking for an opening to Müllerian structures.

The assessment of the internal genital anatomy was commenced by means of urethroscopy, looking for a dorsal connection to a Müllerian structure. This was followed by an internal inspection of the pelvic organs, which in the early years of this study was done by laparotomy via a Pfannenstiel incision, but since 1994 has been done by means of laparoscopy. In both methods Müllerian structures and gonads were looked for in the pelvis and where necessary, bilateral gonadal biopsies were taken for histological evaluation.

RESULTS

The results are tabulated against the subsequently proven diagnoses. The diagnoses and number of patients are as shown in Table 1.

Table 1. Diagnosis of 267 patients investigated for ambiguous genitalia and number of each type

XX-Disorder of Sex Development.	XX-DSD	66
OT- Disorder of Sex Development.	OT-DSD	111
XY-Disorder of Sex Development.	XY-DSD	14
Mixed Gonadal Dysgenesis.	MGD	10
Syndromic.	Syndr.	17
Undervirilized males	UGS	49

Clinical features

The clinical feature that often led to the child's gender being questioned was the penis. Here it was either not in keeping with the assumed gender, or it was inappropriate for age of the child assumed to be male. The following table (Table 2) gives the sizes fitted into the child's ultimate diagnosis.

Other than an ambiguous penis, the perineal orifices were often ambiguous as well. There were 25 patients who showed separate urethral, vaginal and anal orifices. In the remaining patients the perineum showed a single urogenital orifice, together with a normal anus.

The labio-scrotal folds in patients showed a full array from normal bilateral labia (93 patients), to a mixture of labia and hemiscrotal folds (56 patients), bifid hemiscrotal folds (84 patients), or a male-like fused scrotum (34 patients).

Table 2. The Penile size of 267 clinically assessed patients

Penile size (Normal value)	XX-DSD	OT-DSD	XY-DSD	MGD	Syndr.	UGS
Large (>3.5cm)	11	2	-	-	-	1
Normal (2.5-3.5cm)	15	46	5	1	4	19
Small (1.0-2.5cm)	30	60	6	7	5	29
Clitoris (<1.0cm)	10	3	3	2	8	-

Biochemical findings

All children had a serum electrolyte assay. The serum sodium ranged between 133-144 (mean = 137 mmol/l) and the serum potassium ranged between 2.7-5.8 (mean = 4.1 mmol/l). No diagnostic biochemical patterns, or salt losing enteropathies were found among these patients.

Serological studies

Serum steroid studies were recorded for patients with XX- and OT-DSD conditions only. In 39 patients the elevated 17-ketosteroid precursors of cortisol were diagnostic of XX-DSD. Assays were done in 36 patients with OT-DSD showing a wide range of serum levels of cortisol, progesterone, ACTH and DOC, but failed to show any diagnostic pattern. Testosterone levels and response to βHCG stimulation were found to decline with increasing age (See Chapter 9).

Chromosomal assays

The standard method of assaying chromosomal structure looking for abnormalities was applied in 113 patients. Of the patients tested in this manner, 86 (76%) patients had a normal 46,XX karyotype, and 20 (17%) patients had a normal 46,XY karyotype. Seven patients had Mozaic / Chimeric karyotypes (Table 3). HY-Antigen positivity was not tested for in any of the patients.

Table 3. Chromosomal make-up of 113 patients

Chromosomal structure	XX-DSD	OT-DSD	XY-DSD	MGD	Syndr.	UGS
46,XX	18	68	0	0	0	0
46,XY	0	7	3	2	1	7
Mosaic/Chimeric	0	3	0	2	2	0

Imaging techniques

An ultrasound and genitogram were only done in the early years of this study, and results here only reflect those early patients. In all, 17 patients had an ultrasound of the pelvis, and in nine (52%) a Müllerian system was identified. Ultrasound investigations falsely indicated that Müllerian structures were present in three patients.

A genitogram was done in 25 patients. This correctly showed the presence of a Müllerian system in nine patients (36%), but failed to show a Müllerian system in 16 patients, who at subsequent endoscopy or laparotomy were shown this was to be present.

Internal genitalia

A urethroscopic and laparotomy or laparoscopic examination was done under general anaesthesia, specifically to evaluate the internal genitalia in 206 patients. Twenty five patients had separate urethral, vaginal and anal perineal openings, 24 of whom had an internal inspection and the 25th patient did not. Of the remaining 182 patients examined, 131 had a urogenital sinus where the Müllerian system joined the urethra to open onto the perineum, and in 51 patients no Müllerian structure was found. The combined findings of the external and internal genital examination demonstrated the status of the Müllerian system in 207 patients, as shown in Table 4.

The pelvic examination also showed that 88 patients had a uterine structure. In 42 patients the uterus was of an appropriate size for age, and the remaining 46 patients either had a rudimentary or hemiuterus.

The finding of either a vagina or uterus did not predict the presence of the other. Thirty four patients had a vagina present without a uterus. Similarly there were ten patients who had a uterine structure without the presence of a vaginal opening.

Table 4. The position of the Müllerian system in 207 patients

Müllerian opening	XX-DSD	OT-DSD	XY-DSD	MGD	Syndr.	UGS
Perineal Müllerian opening	9	8	0	0	8	0
UroGenital sinus	17	88	11	2	2	11
Nil	1	15	1	6	0	28

Gonadal position

The gonadal positions were recorded in 208 patients. Only a single gonad could be found in ten patients, accounting for 406 gonads. In 140 patients the gonads were situated bilaterally in the pelvis, inguinal canal or scrotum. In the remaining 58 patients the gonads were found in varied positions, with one gonad in either the pelvis, inguinal, scrotal position and the other gonad in a different position. The gonadal positions are shown in Table 5.

Table 5. Gonadal position as found in 208 patients

Gonadal position	XX-DSD	OT-DSD	XY-DSD	MGD	Syndr.	UGS
Pelvic	44	152	10	7	17	24
Inguinal	0	17	7	3	1	31
Labio/Scrotal	0	48	11	9	0	25
Missing	0	5	0	1	0	4

Gonadal biopsies

Not all gonads were biopsied for histological assay. Gonadal biopsies were only done when the gonad looked abnormal or not in keeping with the genital structures i.e. in 11 XX-DSD, 111 OT-DSD, 12 XY-DSD, 5MGD, 12 Syndromic and 33 UGS patients. Where the gonad was present in the labio-scrotal fold, the biopsy was often done through a scrotal or inguinal route, whilst a laparotomy was done to establish the nature of the internal genital structures and to biopsy all pelvic gonads (Table 6).

Table 6. Histological combinations of gonads in 184 patients

Gonadal biopsies	XX-DSD	OT-DSD	XY-DSD	MGD	Syndr.	UGS
Testes bilateral	0	0	12	2 (+3 Testis-Amorphous gonad comb.)	1	29 (+4 Single Testes)
Testis + Ovotestis	0	16	0	0	0	0
Testis + Ovary	0	24	0	0	0	0
Ovotestes bilateral	0	31 (+5 single Ovotestes)	0	0	0	0
Ovotestis + Ovary	0	35	0	0	0	0
Ovaries bilateral	11	0	0	0	11	0

Gender assignation

115 patients were primarily assigned the female gender, often by the parents prior to admission or following our investigations. Similarly 152 patients were assigned the male gender. In total 18 patients (19%) had a change in the assigned gender. Eight patients changed from an initial female gender to male and ten from male to the female gender (Table 7).

Table 7. Assigned and changed gender of 267 patients

Gender assignated	XX-DSD	OT-DSD	XY-DSD	MGD	Syndr.	UGS
Primarily Female	44	51	4	0	14	2
Primarily Male	22	60	10	10	3	47
Gender changed	3 F to M	3 F to M 9 M to F	2 F to M 1 M to F	0	0	0

DISCUSSION

Disorder of Sex Development is not a common group of conditions among the general population. Of these, XX-DSD is generally regarded as the commonest DSD condition in the World.[1]

This study showed that the external and internal anatomy in patients with ambiguous genitalia bear no relation to the underlying gender or cause of that condition. The presence of a natural vagina or penis, however give an indication of the potential gender in which these children could be raised in the future.[2]

An analysis of the available investigations show that typical steroid chromatographic results are useful investigations to identify the XX-DSD patients.[3] Testosterone levels decrease with age in children with OT-DSD.[4,5] Neither of these investigations are diagnostic and in an area where XX-DSD is not the common cause of DSD, early and more invasive investigations have been suggested and are indicated to get to a diagnosis.[6]

The ultrasound and genitogram assessments in the correct settings may see the internal genital structures, but locally were not found to be helpful in making a diagnosis.[6,7] Both investigations are dependent on the patient's age and the operator's experience and skill. The older the patient and the more experienced the operator, the more reliable the results became.

On the basis of the diagnostic reliability of each investigation on this cohort with ambiguous genitalia, an investigative protocol was formulated. This allowed the cause of the ambiguity and type of DSD to be established expeditiously and with some certainty.

The protocol consists of three quick procedures, i.e. a chromosomal assay, urethroscopy and laparoscopic gonadal biopsy. A chromosomal assay would assess the genetic tendency of the child's gender. Secondly, urethroscopy examines for the presence of a Müllerian system, and finally a laparoscopic pelvic examination establishes the presence of an internal Müllerian system and allows gonadal histological sampling, (Table 8). Once the type of DSD was defined, more specific tests e.g. steroid chromatography etc. could be done to define specific types.

Table 8. Essential investigations for a diagnosis

Diagnostic investigations	XX-DSD	OT-DSD	XY-DSD	MGD
Chromosomes	XX	XX / XY / Mozaic	XY	XY
Urethroscopy & laparoscopy	Vagina & Uterus	? Vagina & ? Uterus	? Vagina	Nil
Gonadal histology	Ovaries	Ovary, Testis or Ovotestis	Testis	Streak Testis

Regarding the establishment of a diagnosis on this cohort of patients, despite the records of our 267 patients with ambiguous genitalia being incomplete, it was possible to make a diagnosis in 262 patients. There were five syndromic patients without a definite diagnosis, but had an ill defined form of DSD. There were 49 undervirilised or severe hypospadiac patients who were strictly speaking male patients, although they required investigation for ambiguity of the genitalia, they fall out of the group of DSD.

Calculating the incidence of the various DSD types was therefore based on the 218 patients who had a DSD. OT-Disorder of Sex Development was seen in 111 of the 218 DSD patients in this study, which makes this the single commonest cause of DSD (51%) among the local black African patients presenting with ambiguous genitalia. This high incidence of OT-DSD is different from that reported elsewhere, but confirms the incidence in previously published articles from South Africa.[4,8,9]

The diagnosis of OT-DSD, is dependent on the histological finding of the presence of ovarian follicles or oocytes as well as seminiferous tubules, representing ovarian and testicular tissue, both seen in the same patient.[10] The need for gonadal histology in making a diagnosis of OT-DSD and the frequency this condition is seen locally, requires an investigative protocol different from elsewhere.

Gender re-assignment is best done in the young child, as was the case in 18 patients.[11] In several patients who should have had a reassignment of gender, the choice was not to do so, as the child was considered too old to change the gender socially(> 1 year of age), or parents wished the child to maintain the original gender. Early investigation and diagnosis allows for easier acceptance of gender change.

CONCLUSION

OT-DSD is the commonest single cause of DSD seen in our practice. The external and internal anatomy bear no relation to the underlying gender or cause of this condition.

A standard protocol of chromosomal assay, examination of the genitalia under anaesthesia and an internal genital assessment with gonadal biopsy allows for an expedited diagnosis in all patients. Early investigation and diagnosis allows for easier acceptance for gender change.

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Chapter 4

OvoTesticular Disorder of Sex Development in Southern Africa: the clinical picture

This chapter is based on Wiersma R. True hermaphroditism in Southern Africa: The clinical picture.

Pediatr Surg Int. 2004; 20(5):363-368.

ABSTRACT

This article was originally published as an 18-year retrospective review of 85 patients with OvoTesticular Disorder of Sex Development (OT-DSD), with the aim of facilitating early recognition of this condition. For the purpose of this thesis, this study has been up-dated to include all 111 patients seen and investigated up to and inclusive of 2006.

The diagnosis of OT-DSD requires a high index of suspicion for subtle genital anomalies. Although there were no pathognomonic clinical features, the child is likely to have a normal-to-small for age penis, bifid labio-scrotal folds, a perineal hypospadias and in 53% of patients there was a palpable gonad.

This paper highlights the range of clinical features seen in patients with OT-DSD in this region, as well as some of the management dilemmas associated with OT-DSD in a Third World population.[1]

INTRODUCTION

In general practice worldwide, Disorders of Sex Development (DSD) is an uncommonly seen condition. Although the proportions of the individual conditions making up the spectrum of DSD changes from country to country, OT-DSD is universally regarded as a rare condition, constituting between 3 and 10% of the total.[2]

In Southern Africa, however, this condition is seen more frequently.[3] The majority of patients (42%) who presented with ambiguous genitalia were found to have OT-DSD.[4]

This chapter reviews the presentation, clinical features and results of investigations in patients seen with OT-DSD, with the aim of facilitating early recognition of this condition.

PATIENTS AND METHODS

A retrospective review was made of all patients diagnosed with OT-DSD in the Paediatric Surgical unit of the University of KwaZulu-Natal, Durban, South Africa. Over a 23-year period (1984-2006 inclusive), 111 patients were seen with OT-DSD, their ages ranging from the newborn to 13 years of age. Fifty-six patients were 6-months or younger at the time of admission. All race groups were represented among the patients who were investigated for ambiguity of the genitalia, however, all OT-DSD patients were black Africans, with the exception of two children of mixed racial origin.

Of this cohort, 80% patients were referred from peripheral Southern African hospitals (i.e. rural), and the remaining 20% patients were referred from the greater metropolitan hospitals (i.e. urban). There was no geographical area, nor were there any family groups with a particularly higher incidence.

All patients were referred with problems of the genitalia. Sixty-nine patients (78%) patients were referred specifically with ambiguity of the genitalia. The remaining 42(22%) patients were referred as males, 32 with severe hypospadias and associated undescended gonads, and ten patients as undervirilised males with a micropenis.

At presentation patients underwent a clinical assessment in conjunction with serological, chromosomal and steroid assays. This was followed by a full examination under general anaesthesia, specifically looking at the status of the phallus, labio-scrotal folds, position of gonads, perineal orifices and any secondary sexual characteristics. These features fullfil the Prader Classification of sex disorders.[5] A urethroscopy and a full evaluation of the internal genitalia, including a biopsy of both gonads completed the assessment.

For clarity, the term 'phallus' is used to describe the common structure seen in both the male and female foetus. This structure, as a result of androgen stimulation, enlarges to form the 'penis'. The normal male has a penis >2.5cm long at birth.[6] Without such androgen stimulation, the phallus remains rudimentary, constituting a 'clitoris'. In this thesis the distinctive sizes used for the clitoris is <1.0cm in stretch length, a small penis is between 1.0-2.5cm stretch length, a normal penis is 2.5-3.5cm stretch length and a large penis is >3.5cm stretch length.[6,7]

The differences between labia and hemiscrotal folds are that the latter are prouder structures, generally caused by distension of either a gubernaculum or gonad, and have rugae.

The internal genitalia were inspected first via cystoscopy, looking at the posterior wall of the urethra for remnants of the Müllerian system. This was followed with an internal abdomino-pelvic examination. The pelvic organs were examined and gonadal biopsies were taken when these looked discordant or structurally abnormal. Biopsy material was sent for histological examination.

RESULTS

Clinical features

The clinical features of children with OT-DSD constituted a genital spectrum, with the common feature among all 111 patients being that none looked like a totally normal male or female. All patients had some genital oddity, however subtle, that made the parent or medical attendant query the child's gender.

The external genitalia in the child without secondary sexual characteristics were a permutation of four genital features. These were the:

- Phallus
- Labioscrotal fold(s)
- Gonads
- External perineal orifice(s).

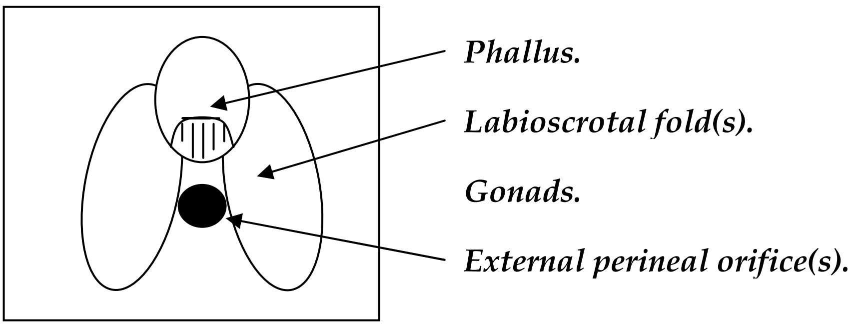

Figure 1. Schematic presentation of 4 genital features

Phallus

The most visible feature that gave rise to the genital uncertainty was the penis. Of the 111 patients there were 108 children with a penis and three with a clitoris proper. In only 14 patients did the phallic structure match the labio-scrotal folds enough to give a convincing appearance of either gender (5 males & 9 females). Of those children with a penis, 47 had an appropriate for age male structure, as seen in Table 1.

Despite the tendency of this group of children to have a penis, only half (n=60) were thought to be 'males' by their parents and the other half (n=51) 'females'. Among those 60 children thought to be males, only five patients looked like a male at a cursory inspection of the genitalia, despite the fact that 27 patients of this cohort had an appropriate for age penis. The remaining patients either had an abnormally large penis (n=1), a small for age penis (n=31), or a clitoris (n=1). There were 51 patients who were thought to be females. Here only nine children looked like a female child at first glance, with labia and either a clitoris or short penis. There were 2 children who had a clitoris and the remaining 49 children had a spectrum of penile sizes, i.e. 1 large, 20 normal and 28 short for age sized penises.

Table 1. Phallic structures in 111 patients

Penile size		Patient numbers
Large	(>3.5cm)	2
Normal	(2.5-3.5cm)	47
Small	(1.0-2.5cm)	59
Clitoris	(<1.0cm)	3

Labio-scrotal folds

Looking at all 111 patients there was a range of labioscrotal folds, commencing with the nine patients who appeared to look like 'normal females' at a cursory glance, with bilateral labia, a clitoris or small penis and separate perineal openings (Figure 2). These

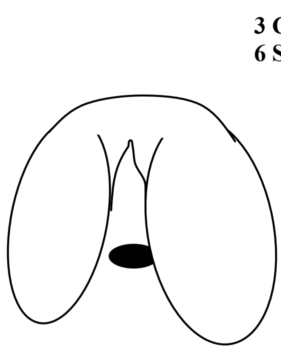

Figure 2. Schematic presentation of the external genitalia in 9 patients with a female appearance

children were investigated due to one labium being larger than the other, which did not look like a hemiscrotal fold. In total there were 26 children with bilateral labial folds, associated with a range of penile sizes (Figure 3).

There were 76 patients with some scrotal appearing structure, either as a hemiscrotal fold (n=20, Figure 4), bifid scrotal folds (n=47, Figure 5), or as a fused scrotum (n=9, Figure 6). These were seen with a range of penis sizes.

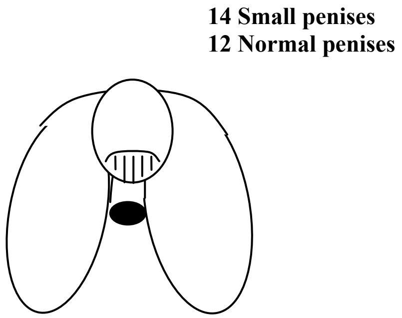

Figure 3. Schematic presentation of the external genitalia in 26 patients with bilateral labia

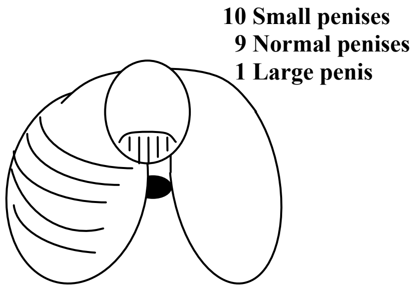

Figure 4. Schematic presentation of the external genitalia in 20 patients with one labium and one hemiscrotum

Forty-seven patients had bifid scrotal folds, usually with a more ventrally positioned urethral orifice and a variance of accompanying chordee (Figure 5)

Only nine 'male' patients had a normal scrotum. The scrotum was seen in combination with a hypospadic penis, the hypospadias was classified as scrotal (n=3), peno-scrotal (n=4) and penile (n=2). The penis-scrotal relationship was as shown in Figure 6.

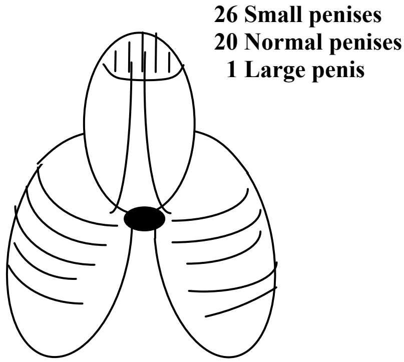

Figure 5. Schematic presentation of the external genitalia in 47 patients with bifid scrotum

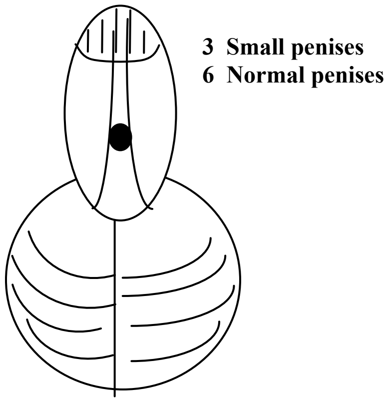

Figure 6. Schematic presentation of the external genitalia in 9 patients with penis and scrotum

Gonads

Gonads were palpable in 53 patients. Fifteen of these patients had bilaterally palpable gonads and in 38 there was a single palpable gonad in an inguino-scrotal position, with the contralateral gonad subsequently found in the pelvis. In two patients there was a single pelvic gonad only and in the remaining 56 patients bilateral gonads were found within the pelvis.

Urethral orifices

In the 111 patients investigated, the position of what looked like the urethral orifice showed considerable variation. Eleven patients had the opening on the penis (3 glandular, 8 penile) in conjunction with a small penis in five and an appropriate for age penis in six patients. Five of these patients had a vaginal structure. Ninety-two patients had the 'urethral opening' in a peno-perineal, perineal or scrotal position. These external openings constituted a 'urogenital sinus', connecting a urethra and vagina internally in 88 patients.

The remaining eight patients had visibly separate genital perineal openings i.e. a separate urethra and vagina. One patient had this in relation to a normal clitoris, six others with a small penis as well as one with a normal male penis. Three patients who had a clitoris or small penis and separate perineal openings were investigated because they had one labium larger than the other.

Secondary Sexual characteristics

There were only three children who presented with secondary sexual characteristics. They were aged ten, 11 and 13-years, all of whom had bilateral breast development without receiving hormonal replacement. No child presented with urethral bleeding.

Internal genitalia

On intra-pelvic inspection, only 59 patients were found to have a uterine structure. In 30 patients the uterus was an appropriate size for age structure, but in the remaining 29 patients, the uterus was abnormal consisting of a bifid or hemiuterine structures of abnormal size. In total there were 52 patients who had no uterine structure.

Although 57 patients had both a vagina and a uterus, two patients had a uterus but no vagina and 32 patients had a vagina, but no uterus. Five patients had a utriculus without a uterine structure and in 15 patients no remnants of any Müllerian structures could be found.

There were 93 patients who had intra-pelvic gonads. In 56 patients these were bilateral pelvic gonads and the remaining 37 patients had a single pelvic gonad.

Biochemical findings

All children had a serum electrolyte assay. The serum sodium ranged between 133-144 (mean 137 mmol/l) and the serum potassium ranged between 2.7-5.8 (mean 4.1 mmol/l). The results were all within normal limits and generally unhelpful in the diagnosis of OT-DSD.

Serological studies

Steroid assay was initially done on all patients with ambiguous genitalia. Subsequently, as OT-DSD appeared to be common, and the steroid assay findings were non-specific, this investigation was only done where the child did not prove to be a OT-DSD.

Incomplete steroid assays were done in 47 patients with OT-DSD, and showed some abnormality in the testosterone level and normal values of all other steroid levels, e.g. Serum cortisol (mean 370 mmol/l; range 85-1195 mmol/l). The "Random Basal Testosterone" and "3-day Basal Testosterone" test following ß-HCG stimulation were done in 10 patients, of whom 6 had a good and 4 a poor response. Of the six patients who had a response showing an increase in level to a mean 6.8 mmol/l (2.7-12.8mmol/l) or seven times increase in concentration, five were children <7 months of age and one was 13 years. The other four patients who had a poor response to the ß-HCG stimulation, had an increase of less than 0.78 mmol/l (range 0.70-1.00 mmol/l), and were generally older than one-and-a-half years.

Chromosomal assays

The standard method of assaying chromosomal structure looking at 20 fields was applied in 78 of our patients. Of those patients tested in this manner, 68 (89%) patients had a normal 46XX chromosomal pattern and seven (11%) patients had a normal 46XY chromosomal pattern. Three patients were found to have a chimeric or mosaic karyotype. HY-Antigen positivity was not tested.

Histology

Gonadal biopsies were done in all 111 patients. Five patients only had a single gonad, an ovotestis. In 59 patients no external gonad could be palpated, requiring a laparotomy or laparoscopy to view and biopsy such gonads.

In 52 patients, one or both gonads were found in an external position, of these 15 patients had bilateral palpable gonads in a scrotal (n=7), inguinal (n=5) or inguino-scrotal (n=3) position, requiring a local incision for the biopsies of both gonads.

The biopsies of the gonads showed there were 118 ovotestes, 59 ovaries and 40 testes. In total, 31 patients had bilateral ovotestes (Table 2).

Eighty percent of ovotestes were of a histologically mixed type. The composition of these gonads varied considerably. The histology of these gonads is reported in Chapters 5 and 6.

Table 2. Histological type and position of 217 gonads biopsied

Gonadal position	OvoTestes	Ovaries	Testes
Pelvic	78	51	20
Inguinal	12	2	3
Scrotal	28	6	17

DISCUSSION

The high incidence of OT-DSD in the Southern African black population group is unusual. Despite considerable research on the condition, no explanation for this occurrence has yet been offered.[1,3,8,9] Research in our own unit has shown the local incidence of OT-DSD among the black African paediatric patients presenting with ambiguous genitalia to be as high as 51%.[1] It is of note that if the patients with OT-DSD are excluded, the incidence of the various other causes of DSD are similar to those reported elsewhere.[10,11]

Serving a largely rural, poor population implies that decisions on management need to reflect the importance of the family unit, the customs, and the lack of social services. Equally one needs to be aware that hospital follow-up will be irregular and costly to the family, due to transport and other socio-economic factors. As the upbringing in these communities is gender based, an early decision on the gender of rearing is essential and assists the future management of the child.

The presenting features of OT-DSD to the general practitioner are age dependent. During the neonatal period and infancy, the presentation was to help define the child's gender. In the older child, medical advice was sought for problems of abnormal secondary sexual characteristics e.g. where penile growth was excessive or inadequate, breast enlargement or sexuality problems.

Recognition of the child with genital ambiguity requires the examiner to be suspicious of genitalia that are other than normal. At first glance five children looked like normal males and nine like normal females, but even these patients on closer inspection revealed some genital oddity. The diagnostic clinical features that distinguish patients with OT-DSD are the abnormal combination of phallus, labio-scrotal folds and palpable gonads. There was a noticeable trend on examining these children, where the smaller phallic size was associated with a more feminized perineum. The labio-scrotal folds here appeared more like labia and were less likely to contain gonads. Conversely, the larger the phallus, the more scrotal in appearance the folds became and the gonads were likely to be palpable. Palpable gonads are likely to contain testicular tissue, as shown by the descended gonads in this series.

The assignment of gender remains a difficult, but an important issue, particularly in a society where the upbringing is totally gender based. The older children in our series (aged >10 years) "knew" which gender they wished to be brought up in, but in the neonate or infant, constituting 52% of children under six months of age in our cohort, assigning a gender was made more difficult, because there was no 'back-ground' gender on which to base this decision. The difficulty in choosing the gender that is best suited for these children is therefore inversely proportional to the age.

The gender of choice is influenced by an array of factors, the first of these is that patients with OT-DSD are essentially infertile.[11] Dealing with young children therefore allows the choice of best-suited gender to be made, but in reality only a few children changed gender. The originally chosen gender was retained in 99(89%) of these children, and in only 12 children, three of whom were over 1-year of age, was the gender changed. In only three patients was the change from the female to the male gender.

The gender of rearing should be based on the parental and older child's wishes, together with medical advice based on the internal and external presenting anatomical structures and functional gonadal tissue. This maybe contrary to some modern opinion where a 'neutral gender' is suggested until the child can choose for it self.[12]

It was odd that only 54% of children were thought to be males by their parents, particularly among the black African people where the male child is the preferred gender.[1] Although the majority of OT-DSD children had a penis, we found that when parents were left to make the difficult decision of gender unaided, the feature they looked for was the presence of a scrotum, rather than the phallus (unpublished data). All children with a fused scrotum were thought to be 'male', this was irrespective of the size of the phallus. Alternatively, all children who were initially labeled as female, had bilateral labial folds, despite the fact that several had a normal or large male-like phallic shaft.

The investigations of patients with DSD are directed toward defining the underlying condition. Although OT-DSD is locally common, the normal investigations for Disorders of Sex Development, e.g. assessment of salt losing states, chromosomal make-up and steroid chromatograms, should proceed to identify other causes of DSD. These investigations are, however, non-contributory in the diagnosis and further management of the child with OT-DSD. Here there are no characteristic electrolytic, chromosomal or steroid profiles. The diagnosis of OT-DSD can only be made on gonadal biopsy. With our locally high incidence of this condition (51%) and in the absence of specific diagnostic features, gonads of all children presenting with ambiguous genitalia need to be biopsied. The histology of the gonads and the inspection of the internal genitalia under these conditions form an essential part of the initial DSD evaluation and are of help with the further management of these patients.

Examination of the internal genitalia in small children by radiographic and ultrasound investigations is difficult, and in developing countries where radiological skills are scarce, such studies are often unreliable. A combination of urethroscopic and laparoscopic inspection of the child at the same session, proved to be the most accurate assessment of the internal genitalia.[1,13]

Evolving clinical features and impending pubertal development may mandate further investigation in the OT-DSD. The development of breast tissue or per urethrum bleeding etc. would require the investigation for and the removal of any occult ovarian tissue. Similarly, clitoral growth in a child brought-up as a female requires that discordant testicular tissue be removed.

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Chapter 5

The gonads of 111 South African patients with OvoTesticular Disorder of Sex Differentiation

This chapter is based on Wiersma R, Ramdial PK. The gonads of 111 South African patients with OvoTesticular Disorder of Sex Differentiation.

***J Pediatr Surg*. 2009; 44(3):556-560.**

ABSTRACT

This was a retrospective study, which looked at the clinical findings, internal genital assessments, and the histology of all gonadal biopsy specimens taken from patients diagnosed with OvoTesticular Disorder of Sex Development (OT-DSD) over a 23 year period.

Seventy complete ovotestes, ovaries and testes were completely excised and submitted for histology. Three distinct ovotesticular types are identified in the Southern African patient with OT-DSD, which have not been described previously. The structure of these gonads has bearing on the type of biopsy done and the subsequent management of the ovotestes.

The aims of this paper were to describe the gonadal tissue found in the Southern African patient with OT-DSD.

INTRODUCTION

Ovotesticular Disorder of Sex Differentiation (OT-DSD) is an uncommon cause of Disorder of Sex Differentiation (DSD), and has an estimated world incidence of 4%.[1] With an unusually high incidence of this condition among South African patients investigated for ambiguous genitalia (51%), several authors have shown that OT DSD in Southern Africa is different in several respects**.**[2,3]

As one of a range of DSD conditions, OT-DSD has few diagnostic pointers.[3,4] The diagnosis needs to be confirmed histologically, and gonadal biopsies are therefore a necessary part of the investigations. The pathognomonic histological feature of OT-DSD is the presence of seminiferous tubules and ovarian follicles or oocytes, representing testicular and ovarian tissue, both seen in the same patient.

The single commonest gonad seen in such patients is the ovotestis, which combines these two gender opposite tissues in a single gonad.[3,4] Whilst the histological description of the common ovotestis in the literature suggests a bipolar structure, in Southern Africa the ovotesticular histology is at variance with that description.[5,6]

We describe a complete clinical, genital and gonadal assay in a series of Southern African patients managed for their OT-DSD, and encountered a unique histological pattern of ovotestes.

The aim of this study was to describe the gonadal tissue seen in the Southern African patient with OT-DSD.

PATIENTS AND METHODS

The records of all patients diagnosed with OT-DSD seen by the Department of Paediatric Surgery, Durban, South Africa, over a 23-year period (1984-2006 inclusive**),** were reviewed retrospectively.

Patients had a full clinical, laboratory and internal evaluation. The latter included urethroscopy, inspection of the internal genitalia, as well as bilateral gonadal biopsies.[6] The further management entailed psychological assistance of the family unit, endocrine support with excision of ovotestes and gonads discordant with the gender of rearing.

All gonadal tissue was submitted for routine formalin fixation, and was processed overnight in a Shandon processor. Excision of entire gonads necessitated dissection of the specimen, and separating tubular structures from the gonads. These were processed in separate tissue cassettes. Following fixation, the tissue was embedded in molten paraffin wax and allowed to solidify. The entire paraffin wax block was sectioned at 3 µm thickness and stained with haematoxylin and eosin for histopathological appraisal.

RESULTS

Clinical

One-hundred-and-eleven consecutive OT-DSD patients were managed during the study period. The patients' ages at presentation ranged from 1-day to 13-years. Eighteen patients were older than two years.

The stated reason for referral was ambiguity of the genitalia in 69 patients, hypospadias in 32 patients, and micropenis in 10 patients. Clinically all patients had features requiring investigation for DSD, i.e. abnormal penis, perineal hypospadias, gonadal maldescent, and bifid labioscrotal folds, either as a single or combination of pathologies.

Examination under anaesthesia and internal genital examination of these children showed that 57% of patients had a small for age phallic structure and a Müllerian structure. These features are charted against their external appearance according to the Prader classification, seen in Table 1.[7]

Table 1. Clinical findings of genital structures

Prader Classification	Patient No.	Penile size	Vagina present	Uterine body present	Gonadal position for group
1	9	Clitoris 3	3	2	Pelvic
		Small 6	6	5	
2	27	Small 14	10	5	Pelvic &
		N male 12	9	9	Inguinal
3	20	Small 10	9	2	Pelvic,
		N male 9	6	6	Inguinal,
		Large 1	1	1	Scrotal
4	47	Small 26	23	14	Pelvic &
		N male 20	18	13	Scrotal
		Large 1	1	0	
5	9	Small 3	1	1	Pelvic &
		N male 6	2	1	Scrotal

Key to penile size is as follows: clitoris < 1cm, small penis 1-2.5cm, normal male penis 2.5-3.5cm, large penis >3.5cm

Investigations

The diagnosis of OT-DSD was made on the histology of 217 gonadal biopsies. Five patients were found to have a single gonad only. The positions of the gonads are indicated in Table 2.

The histological examination showed that there were 118 (54%) ovotestes, together with 59 ovaries and 40 testes. Ovotestes were seen bilaterally in 31 patients. The gonadal combinations and age that these gonads were biopsied are shown in Table 3.

Only 52 of the 86 patients with ovotestes had these completely excised, yielding 70 complete ovotestes.

Table 2. Position and Type of 217 Gonads

Gonadal position	OvoTestes	Ovaries	Testes
Pelvic	78	51	20
Inguinal	12	2	3
Scrotal	28	6	17

Table 3. Gonadal combinations and Age at which these were biopsied

Right Gonad	Left Gonad	No. & Age at < 2 years	Gonadal biopsy > 2-years	Total number patients
OvoTestis	OvoTestis	23	8	31
OvoTestis	Nil	3	1	4
Nil	OvoTestis	1	0	1
OvoTestis	Ovary	18	10	28
Ovary	OvoTestis	7	0	7
OvoTestis	Testis	5	0	5
Testis	OvoTestis	7	4	11
Testis	Ovary	11	6	17
Ovary	Testis	4	3	7

Histology

Gross appearance of the ovotestes allowed these to be differentiated into:

- o Mixed ovotestes 105(89%), which were beige and globular in appearance.
- o Bipolar ovotestes 13(11%), consisting of two clearly distinguishable tissues, where the pale ovarian tissue was on top of a rounder testicular structure.

Histopathologically the gonads were divided into;

- o MixedOvotestes
  Admixed type, constituting 44% of mixed ovotestes
  Compartmentalised type, 56% of the mixed ovotestes
- o Bipolar Ovotestes
- o Ovaries
- o Testes

Mixed OvoTestes

Mixed type of ovotestes were globular structures consisting of an outer layer or mantle of ovarian tissue, within which was an inner core consisting of 2 different tissue patterns, a) the Admixed and b) the Compartmentalised ovotestes. This was independent of whether patients had a 46,XX or 46,XY karyotype (Figures 1,2,3).

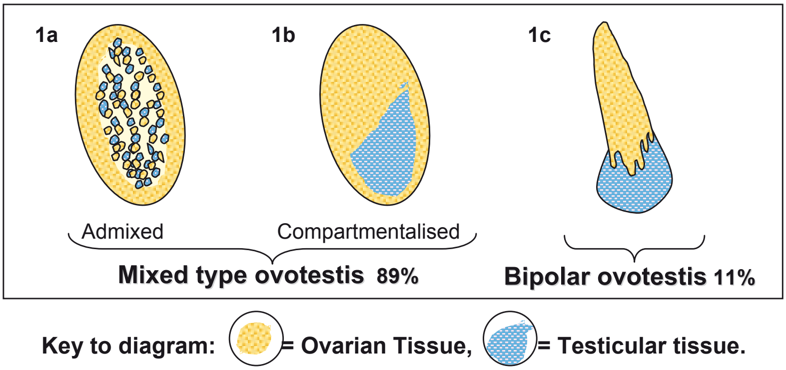

Figure 1 Schematic representation of the ovarian and testicular tissues distribution in the three different ovotesticular types

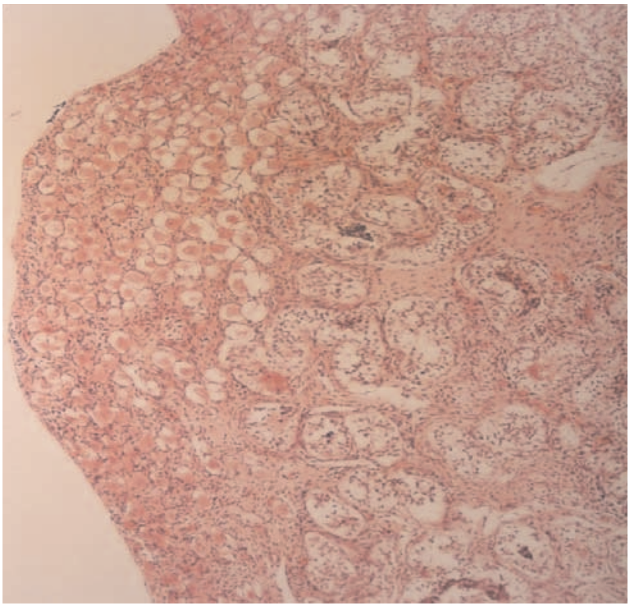

Figure 2. Admixed ovotestis showing ovarian follicles (Lt) and seminiferous tubules (Rt) of photo.

These gonads had an outer mantle of ovarian tissue of variable thickness, surrounding a central core of stroma, containing scattered foci of ovarian and testicular tissue of different sizes. (See Figure 1a) The separate foci of primitive ovarian tissue contained primordial and developing follicles surrounded by stroma. The testicular foci showed primitive testicular tissue comprising immature seminiferous tubules containing spermatogonia and aggregates of Sertoli and Leydig cells, but no spermatozoa were seen among them.

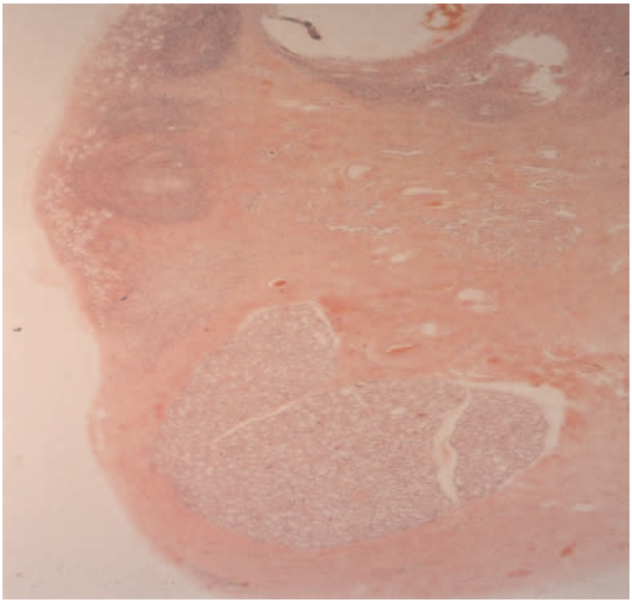

Figure 3. Compatmentalized ovotestis showing an largely ovarian gonad with a lower pole of seminiferous tubules embedded in ovarian stroma.

Here the rim of ovarian tissue completely filled the upper portion of the gonad, and in the lower portion it encapsulated a variable sized core of testicular tissue (See Figure 1b). The tissue ratios ranged from 1:1 to 4:1 ovary:testis. The ovarian tissue contained scattered primordial ovarian follicles. The testicular tissue was composed predominantly of primitive seminiferous tubules containing spermatogonia and lined by Sertoli cells and focal Leydig cells.

Bipolar ovotestes

These gonads showed a strict polar distribution of ovarian and testicular tissue (See Figure 1c). The ovarian component, as the upper pole, consisted largely of primordial follicles as well as focal cystic follicles in an intervening stroma. The testicular component, situated in the lower pole, consisted of immature seminiferous tubules containing spermatogonia and lined by Sertoli cells surrounded by stroma. However no spermatozoa or Leydig cells were seen. There was considerable interdigitation of the upper and lower pole tissues. Fallopian tubular structures were attached in some of these gonads.

Ovaries

The microscopic structure of ovarian tissue comprised of follicles scattered in ovarian stroma. Follicles consisted of numerous primordial and Graafian follicles in varying stages of development. Histologically these ovaries appeared no different from those in the normal female child.

Testes

These consisted of immature seminiferous tubules lined by Sertoli cells, embedded in stroma. Occasional primitive Leydig cells were noted in the mesenchyme surrounding the tubules. No spermatozoa were seen is any testicular material in our patients of any age.

DISCUSSION

OT-DSD is generally regarded as an uncommon cause of DSD and histological descriptions of the gonads of patient with OT-DSD are few.[5] These describe the ovotestis as a bipolar structure with 80% of gonads showing ovarian tissue in a upper and the testicular tissue in the lower pole.[5,8] The two tissues were found in a variable ratio, ranging from a 1:4 ovary:testis, to a 4:1 ovary:testis combination.[5] The ovarian tissue was firm, gritty and yellow, whilst the testicular portion was soft and pink, and a clear demarcation was found to exist between the ovarian and testicular tissues allowing their division.[8]

There are fewer descriptions of the mixed type ovotestis.[9] In these gonads the ovarian tissue is confined to one or more nodules in the hilum of an otherwise unremarkable testis. Only one description was found of a mixed gonad, here there was a diffuse admixture of ovarian and testicular tissue distributed throughout a fibrous stroma.[4]

The locally high incidence of OT-DSD (51%) has lead to a need for histological sampling of the gonads on most patients with DSD. Using minimally invasive surgery to obtain gonadal biopsies has simultaneously provided a composite picture of the gonads and Müllerian system. This method of assessing patients has eliminated the need for ultrasound and MRI investigations.

Our findings of the OT-DSD gonadal histology was based on 111 patients over a 23-year period, and agrees with the available literature in some respects. These being that the majority (78%) of OT-DSD patients have an ovotestis, that the ovotestis was the commonest (55%) gonad in these patients, and that 59% of the ovotestes were situated on the right side of the patient (Table 3). However, our findings were at variance with the literature on the basic histological features. The first of these is that the majority of gonads in the African OT-DSD patient are of a mixed variety (89%), with the remaining 11% being bipolar. The most important feature of the mixed ovotestes is the mantle consisting of ovarian tissue of a variable thickness. This has not been described previously.

The appearance of the mixed ovotestis is that of a globular testis, although the mantle consists of ovarian tissue, making this ovotestis different from previous descriptions. In the compartmentalised type, this ovarian mantle encapsulated the testicular tissues in the lower pole, giving the internal appearance of a bipolar ovotestis. While macroscopically the ovarian mantle makes the admixed ovotestis indistinguishable from the compartmentalised type, here it encapsulates a central core of stroma containing scattered foci of ovarian and testicular tissues. The stimulus for differentiation into these individual types of gonadal tissues can only be speculated on.

Histologically the gonadal tissue was immature, showing ovarian follicles in varying stages of development and testicular tissue without any spermatozoa. This is in keeping with the age group of <2-years of age from whom the gonadal biopsies were taken, (Table 3). Functionally there appeared to be little difference between ovotesticular types, as they lead to the same clinical result. This suggests that they contained similar volumes of 'functional' ovarian and testicular tissue.

The variable thickness of the ovotesticular mantle and the scattered foci of ovarian and testicular tissue in the admixed types, made it difficult to obtain a representative histological sample of gonadal tissue on biopsy. The method of taking gonadal biopsies therefore proved to be crucial, and a longitudinal pole to pole, wedge biopsy, representing the entire length as well as the superficial and deep structures of the gonad was devised, yet leaving much of the gonad intact.[10]

Conservative gonadal surgery should be practiced in these patients where possible.[11,12] This form of management is, however dependent on several factors. Firstly, identifying the gender of rearing at the time of the gonadal surgery, which can often only be done by the child at 6-8 years of age. Secondly is the ability to identify the separate gonadal tissues at operation, and thirdly is the awareness of the 2-4% risk of developing malignancy in the ovotestis or dysgenetic testis in later life.[3,8,13,14] The factors that precluded conservative gonadal surgery in our patients were the mantle of ovarian tissue covering the entire mixed ovotestis, as well as the marked interdigitation of the 2 types of tissues in the bipolar ovotestes, both making separation impossible. In addition one must be mindful of the poor long-term follow-up, for a variety of Third World realities such as cost, distance and tradition. For these reasons locally, the ovotestes are excised.

No reason has to-date been found why there is a higher incidence of OT-DSD in South Africa. Clinically there is little to find. There are a few familial cases reported, and a dated study looking at the genetics in these patients has revealed little.[10,15,16] The fact that this condition constitutes more than half the local number of DSD patients, that they come from all over this country and that their gonads are sufficiently different to warrant a different form of gonadal management, suggests a common underlying genetic lesion.

CONCLUSIONS

In countries where OT-DSD constitutes a significant percentage of the DSD population, gonadal biopsies must form part of the initial DSD work-up.

Three types of ovotesticular structures are seen in the African patient with OT-DSD, a histopathological feature not previously described. Their structure affect the investigations and management of these patients. The mixed ovotestis has an outer mantle of ovarian tissue and an inner core of ovarian and testicular tissue. This precludes conservative gonadal surgery, and to obtain a representative histological sample of this gonad requires a pole-to-pole wedge biopsy.

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Chapter 6

The African OvoTestis: hidden histology

ABSTRACT

The histological records of 52 patients with Ovotesticular Disorder of Sex Development who had gonadal biopsies and gonadal excision, were completely traceable. They formed the cohort of this study comparing the histology of biopsy and whole gonad specimens.

Twenty two gonadal biopsies failed to show the complete histological make-up. Biopsy diagnosis in 11 testes and eight ovaries, were shown to be ovotestes on the completely excised gonadal histology. Three biopsies diagnosed as ovotestes were found to contain only testicular (n=2) and ovarian (n=1) tissue.

The difficulty in obtaining a representative gonadal biopsy reflects on the type of biopsy that is taken and the further management of these patients.

INTRODUCTION

Ovotesticular Disorder of Sex Development (OT-DSD) is the commonest cause of Disorder of Sex Development (DSD) treated by the Department of Paediatric Surgery of the University of KwaZulu-Natal.[1]

The commonest gonad found among these patients was the ovotestis (54%), and their histological structure was locally not found to conform to the bipolar pattern described in the literature.[2,3] Eighty nine percent of ovotestes studied in our department were found to consist of an irregular mixture of ovarian and testicular tissue.

This study was done to compare the histology of gonadal biopsies with the subsequently excised whole gonads, with the aim of determining the representivity of the biopsy samples.

METHOD

The investigation of patients with DSD, in a population where OT-DSD constitutes 51% of all DSD patients, involves taking histological samples of each gonad for diagnostic purposes and management decisions.

On the basis of the sampled histological make-up, gonads that were found to be ovotestes or discordant with the gender of rearing, were excised and send for total histological analysis. This result was then compared with the histology of the original biopsies.

RESULTS

One hundred and eleven patients were diagnosed OT-DSD on the basis of their (bilateral) gonadal histological results. In total there were 217 biopsy samples, as five patients only had a single gonad. The histology showed there were 118 (54%) ovotestes, 59 ovaries and 40 testes.

Based on this histology and distribution, the patients were classified as Lateral, Unilateral or Bilateral OT-DSD[3] :

- o *Lateral OT-DSD* [LTH], i.e. a patient with an ovary on the one side and a testis on the contralateral side, was seen in 24 (21%) patients.
- o *Unilateral OT-DSD* [UTH], i.e. an ovotestis on one side and an ovary (n=35) or a testis (n=16) on the opposite side, was seen in 51 (46%) patients.

- *Bilateral OT-DSD* [BTH], were patients only have ovotestes, as was seen in 31 patients with bilateral ovotestes, and five patients who only a single ovotestis. This group therefore constituted 33% of patients.

On the basis of their external, internal genitalia and gonadal histologies, the patient's management was discussed with the parents and the management team (individually or as a group) and a management plan adopted. This included excision of ovotestes and potentially non-functional gonads. The histology of the completely excised gonadal was the compared with the biopsy sample to provide a reference of usefulness of the biopsy method.

Fifty-two of the 111 patients (i.e. LTH=10, UTH=26, BTH=16) had a completely traceable histological record from gonadal biopsy to excision of the entire discordant and ovotesticular gonadal tissue. These patients formed the study cohort. The complete histological results of those 52 patients are shown in Figure 1 The gonadal histological

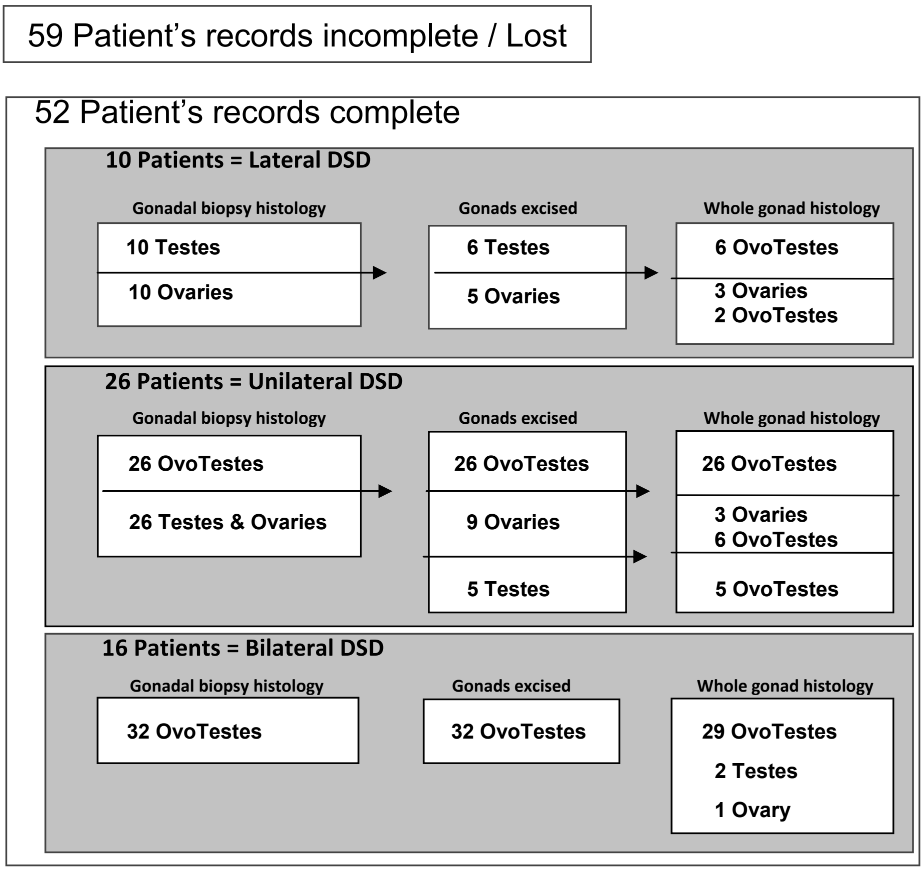

Figure 1. Histological record of biopsy and excision samples

record of the remaining 59 patients was incomplete. Here the original biopsy results are known, but the discordant gonadal tissue has not yet been excised or when excised the results cannot be traced.

Ten patients were classified as LTH on the basis of their 20 gonadal biopsy histologies. Here 11 gonads were excised (6 ovaries, 5 testes). The histology of these 11 gonads showed that all six testes and two of the ovaries were ovotestes. The three remaining ovaries were correctly diagnosed.

Twenty-six patients were classified as UTH. These patients had 40 gonads excised, 26 ovotestes, nine ovaries and five testes. The whole gonadal histology showed that all 26 ovotestes were correctly diagnosed, but the six of the nine ovaries, and all five testes were in fact ovotestes.

There were 16 patients with BTH on the basis of their bilateral ovotestes on gonadal biopsies. Thirty-two gonadal excisions were done, and of these 29 proved to be correctly diagnoses as ovotestes, but in two gonads only testicular and in one only ovarian tissue could be found in the remainder of the excised gonad.

DISCUSSION

This study has highlighted several features of OT-DSD in our setting. Firstly, that the ovotestis is a mixture of ovarian and testicular tissue of variable proportions. This warrants a method of gonadal biopsy other than a simple punch biopsy. This has been made clear by the discrepancies, which have occurred when histological results of the biopsies were compared to that of the whole gonad.

To improve on the representivity of the gonadal biopsies, a longitudinal, pole-to-pole, wedge biopsy has been used when it became apparent that isolated biopsies were not representative.[4] Despite this new method, a truly representative histological picture is still not obtained.

Eighty three gonads were excised from 52 patients and their histological record was a completely traceable. Comparing the biopsy result with the histology of the completely excised gonads showed that 22 biopsies were incorrectly reported. Here 11 testes and eight ovaries on biopsy result should have been reported as ovotestes when more gonadal tissue became available with the whole gonad. More difficult to explain are the three biopsies diagnosed as ovotestes, which on the whole gonad histological analyses only showed testicular tissue in two and ovarian tissue in one. This is slightly different from the original study of fewer patients where the majority of histological misdiagnoses were testes.

The prevalence of ovotestes is greater than originally thought. The original percentage was 118 ovotestes of 217 gonadal biopsies (54%), and if we now look at the 83 whole gonads sampled there were 74 ovotestes (89%). This misrepresentation of gonadal tissue must raise the question how many patients were wrongly diagnosed as under-virilized males with testeThe question whether a the total excision of the OT-DSD gonads is safer than leaving discordant gonadal tissue in such a child remains unanswered by this article.[5] The possible dangers of leaving ovotesticular tissue are those of leaving active ovarian tissue in children who may be raised as males and malignancy in gonadal tissue, especially in the child with a 46XY karyotype.[6,7]

CONCLUSION

This study showed that a large percentage of testes in patients who are OT-DSD were found to be ovotestes when the excised gonad was histologically analysed. The ovotestis appears to be more prevalent then previously thought. This previously hidden histology does have some bearing on the management of these patients. Especially as this study also shows that a considerable percentage of these patients (46%) do not return for follow-up and consequently the discordant gonadal tissue is not removed.

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Chapter 7

Intrapelvic genital evaluation of the child with OvoTesticular Disorder of Sex Development: laparotomy vs laparoscopy

ABSTRACT

Ambiguity of the genitalia is uncommon in children. Although all medical attendants appreciate the urgency of making a diagnosis, the management of these patients varies from country to country.

Under conditions where i) the population is largely rural, ii) OvoTesticular Disorder of Sex Development (OT-DSD) is common and iii) follow-up periods are irregular, establishing an early diagnosis requires an assessment of the internal genitalia and histology of the gonads.

The aim of this chapter was to compare and contrast the two methods of assessing the internal genitalia of children with OT-DSD and obtaining gonadal biopsies.

INTRODUCTION

Ambiguous genitalia is an uncommon presentation in children. The management of these patients varies greatly according to regional prevalence of the condition, the doctor's preference and patient's accessibility to the hospital.

All patients with OvoTesticular Disorder of Sex Development (OT-DSD) from the Southern African region were referred to the Department of Paediatric Surgery at the University of KwaZulu-Natal, Durban, South Africa. Over a period of 23-years, 111 such patients were seen and managed.

OT-DSD is a condition where the diagnosis is dependent on the histological proof of ovarian and testicular tissue in the same patient.[1] Urgent gonadal biopsy and examination of the genital system allowed a rapid diagnosis and decision on management.

Intrapelvic explorations for such assessments and subsequent surgical correction of the Disorder of Sex Development (DSD) condition had in the past been done via a Pfannenstiel laparotomy incision. As minimally invasive surgery filtered through to Southern Africa in the 1990's, children with DSD were starting to be investigated via a laparoscopic assessments of the peritoneal cavity and such methods have appeared in the literature.[2,3,4,5]

The aim of this retrospective study was to compare and contrast laparotomy and laparoscopy as the two methods of internal examination and gonadal biopsy used on patients with OT-DSD.

PATIENTS AND METHODS

Over a 23-year period (1984-2006 inclusive), 111 patients have been investigated for OT-DSD. The patients ages ranged from the newborn to 13 years of age, of whom 56 were six months or younger at the time of admission.

Patients who presented early in the series (1984-1997) had an internal genital assessment via an open Pfannestiel laparotomy. Those patients who presented subsequently (1998-2006) were assessed using laparoscopy. The gonadal biopsy was done at the time of internal inspection. Both methods are described.

Both methods of examination were preceded by a urethroscopy, to visualize any Müllerian structures opening into the urethra. The cystoscope was slowly advanced up the urethra under vision, and the infusate was used to dilate any Müllerian remnant on the dorsal urethral surface. If present, the length of the vagina and the presence of a cervix were noted. Finding the infusate inside the pelvis at subsequent inspection of the internal organs was useful confirmation of patency of the Müllerian system. At the end of this procedure the bladder was completely emptied.

A laparotomy through a Pfannenstiel incision was done on a fully prepared and draped abdomen. This consisted of a suprapubic transverse skin and fascial incision, whilst opening the 'linea alba' longitudinally. The peritoneum was opened on either side of the emptied bladder and the pelvic structures were palpated and inspected. Any bilateral tubular structures or gonads present were delivered through the wound and inspected. As the gonads of the patient with OT-DSD may clinically be indistinguishable from the normal, a pole-to-pole wedge biopsy of each gonad was taken and send for histology.[1] The viscera were returned to the abdomen and the wound closed in layers with absorbable sutures.

In contrast, the laparoscopic examination was preceded by emptying the bladder and stomach. On the surgically draped abdomen, a small umbilical incision was made extending onto the peritoneum, and a purse-string suture placed through which a 5 mm endoscopic-port was inserted under vision.[6] The abdomen was insufflated with CO_2 to a pressure not exceeding 8 mmHg, and the operating table placed in a Trendelenberg position to facilitate the movement of bowel out of the pelvis. Following inspection of the internal organs with the laparoscope, a second and third 5mm ports were inserted bilaterally under vision through the linea semilunaris at the level of the upper lip of the superior iliac crest.

The gonads were grasped with tissue forceps and gently delivered into the 5mm lateral ports under vision. The peritoneum was deflated and the port with gonad delivered outside the abdomen. A pole-to-pole wedge biopsy of the gonad was taken and sent for histology. Grasping the gonad once more, the port was gently rail-roaded over the gonad and tubular structures. The gonad was returned to its original position under vision. The forceps were withdrawn and the wounds closed with a fascial suture. This method of gonadal biopsy has not been described elsewhere.

RESULTS

An internal genital assessment was done in 108 of the 111 patients with OT-DSD. Sixty-one patients who presented early in the series (1984-1997) were evaluated via an open Pfannenstiel laparotomy, and 47 patients who presented subsequently (1998-2006) were assessed using laparoscopy. Three patients did not have an internal assessment early in the study, as they had inguino-scrotal gonads and were found to have no vagina on urethroscopy.

The 61 patients with OT-DSD who had an open laparotomy, had ages ranging from 1-day to 9-years (21 patients <6-months of age). The examination under anaesthesia showed five patients with female genital perineal openings, 47 patients with a normal for age vagina, and nine patients without any vagina.

At laparotomy the internal inspection showed there were 11 patients who had a normal for age uterus. Twenty-two patients had uterine structures ranging from rudimentary to hemi and thin uterine bodies and in the remaining 28 patients no uterine structure could be found. The gonads in 55 patients were positioned, bilateral or singly, in the pelvic. The remaining six patients had external palpable gonads.

During the laparotomy, vision of the lateral tubular structures and gonads was good, but for deep-seated central structures this was less than optimal. Repeat procedures through the same incision were generally not more difficult and tissue planes remained well demarcated. A repeat delivery of the gonad for excision several weeks to months later was occasionally more difficult due to local adhesions. The laparotomy scar remained noticeable in all children. The only complication was superficial wound sepsis, in 3 patients. The mean hospital stay for this cohort was 8.9 days.

There were 47 patients who had a laparoscopic assessment of the internal genitalia and gonadal biopsies. The ages of these patients ranged from 2-days to 13-years, with 22 children being <6-months of age. The primary investigations here showed that three patients had separate female genital perineal openings, 34 patients had a normal for age vagina, and ten patients without any vagina. There were 23 patients where no uterine structure could be found.

At laparoscopy, with clear vision of the pelvis, 37 patients were shown to have either bilateral or singular pelvic gonads. The remaining ten patients had externally palpable gonads.

Visualization of the internal organs by laparoscopy was excellent. Gonadal biopsies were done without difficulty outside the abdomen and repeat procedures were as easy to do as at the first assessment. The remaining scars were small and hardly visible. Wound sepsis of a secondary port site was seen only once, and the mean hospital stay was 3.5 days.

DISCUSSION

The recognition of ambiguous genitalia in children should initiate a series of evaluations to establish the diagnosis and appropriate gender for that child. Ideally this should commence in the neonatal period, before the child has gone home and a gender has been assigned without due care.

Investigation of patients with ambiguous genitalia in the developed world would generally involve a clinical examination and some laboratory investigations, after which the patients will be sent home to await results. Few doctors would suggest an internal examination at that stage, as the likely diagnosis in that environment is XX-DSD on the basis of a 21-hydroxylase deficiency and other less invasive investigation may used.[7] In

Southern Africa, however, 51% of patients have OT-DSD, and the chromosomal make-up and serological assays will neither assist with the diagnosis nor with the further management of the child.[1] For these patients an assessment of the internal genitalia and histology of the gonadal tissue are mandatory from the outset.

To add to the need for internal examination in the management of such a patient in a Third World population, are poverty and living in remote areas. Here hospital reviews become irregular and are costly to the family. The ability to make an early decision on the gender of rearing and future management of the child is of great assistance to the parent and medical staff alike.

Examination of the internal genitalia in small children using radiographic and ultrasound investigations are possible with skilled radiology.[8] In developing countries such radiological skills are variable, and such studies are often unreliable. Under these conditions a combination of urethroscopic and laparoscopic inspection of the child at the same session, proved to be the most accurate assessment of the internal genitalia.[9]

Although visualization of the internal genitalia via a Pfannenstiel laparotomy was accurate, it had limitations with demonstrating midline Müllerian structures. In comparison, the laparoscopic method showed several improvements. There was a panoramic vision of the internal genitalia, partially with the positional removal of bowel from the view, as well as the magnification and clarity of the optical system. The gonads could be properly assessed for position and appearance. When the gonads were brought out through the abdominal wall for biopsy, a more accurate pole-to-pole wedge sample could be taken. The whole laparoscopic procedure was found to be less invasive, allowing the patient to be discharged the same day, and on review there was a less obvious scar in the patient. This method of investigation should be encouraged particularly in the very young.[3]

A simple procedural routine reduced the complications of laparoscopy in the neonates and young children. Emptying of the bladder and stomach, as well as the open method of port insertion allowed safe laparoscopic evaluation in 46 small children. The use of Veress needles in this age group may lead to serious complications.[5,6] Visualization can be achieved with low intra-abdominal pressures, the 8-mmHg pressure used during these procedure had no intra- or post-operative respiratory sequelae. The subumbilical and bilateral abdominal wounds required for the laparoscopic examination were small and healed readily with good cosmetic results. Any post-operative pain caused by either abdominal inflation or wounds was easily controlled with paracetamol.

This was an objective comparison of the two methods for internal genital examination and gonadal biopsy. The findings were that the laparoscopic assessment of patients with DSD was found to be a safe procedure with few complications in all ages.

The comparison of the duration of hospital stay looked a time when patients tended to recuperated in hospital for a lot longer then what is the practice today. This is true both for the developed and Third World country medicine. At the time of this study a mean difference of five days in hospital was noted between the two procedures. The urethro- and laparoscopy combination allowed the infant to feed early and be discharged the same day. However, the lack of transport to the base hospital often stretched the in-hospital stay to a mean 3.5 days, as compared to the 8.9 days for the conventional laparotomy. This allowed the newborn child to go home within the normal time frame and with an appropriate gender that the parents and the medical staff could manage.

Laparoscopy provided an improved method of visualizing the internal genitalia with an adequate access to gonads and genitalia for manipulation, with a shorter hospital stay when compared to open laparotomy.

CONCLUSION

Laparoscopic assessment of patients with OT-DSD was found to be a safe procedure with few complications in all ages. It afforded an improved method of visualizing the internal genitalia with adequate access to gonads and genitalia for manipulation, and a shorter hospital stay when compared to open laparotomy.

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Part 3

The management of patients with OvoTesticular Disorder of Sex Development

INTRODUCTION

This part looks at several general aspects of the management of patients with Ovo-Testicular Disorder of Sex Development (OT-DSD). Patients with this condition have an unusual gonadal type and function, and their gender expression is abnormal. As a result of these and other aspects, an adapted management is required to help these patients, and often their parents, adjust. The four papers that make-up this third part should be seen to complement each other, as each discusses a different aspect of the management of this condition.

The question of what to do with gonads that do not fit in with the gender of rearing is one of the aspects that was studied. The literature provides us with a wide range of treatment models for patients with OT-DSD. This ranges from early complete excision of gonadal tissue together with all necessary cosmetic procedures, to a conservative approach of post-pubertal excision of discordant gonads and late cosmetic procedures. In chapter eight, the surgical management of the local patients with OT-DSD, has been studied. The aim of this study was to look at the functionality of the gonads of these patients, and to validate our management choice.

The early surgical management of patients with OT-DSD was studied holistically in the ninth chapter.

The cosmetic surgical management options for patients with OT-DSD are discussed in the tenth chapter. The care of patients with DSD problems requires a multi-disciplinary team effort. The surgical options should therefore be supportive of the clinical and psychological management of these patients, the timing and need for the correction of some of the ambiguous genital features of these patients are discussed.

Chapter 11 is a discussion on the surgical, ethical and legal considerations of the OT-DSD in the child and adolescent. This partially reiterates some of the surgical procedures discussed in chapters 8, 9 and 10.

Part three emphasizes the need for multi-disciplinary care, extensive counseling and informed consent. It discusses patient and parental need for surgical corrections.

Chapter 8

OvoTesticular Disorder of Sex development: when should inappropriate gonads be excised?

ABSTRACT

Patients with OvoTesticular Disorder of Sex Development have a combination of ovarian and testicular gonadal tissue, and may require surgical management of these gonads. The suggested choice of treatment ranges from early complete gonadal excision to post pubertal conservative discordant gonadectomy.

In the Third World where long-term follow-up is poor, it has been our policy to excise discordant gonads following histological confirmation and a management discussion with all the relevant persons.

The aim of this study was to review our management policy of early excision of ovotestes and all discordant gonadal tissue, as well as look at the factors that were of importance in the shaping of this policy.

INTRODUCTION

Part of the overall surgical management of patients with OvoTesticular Disorder of Sex Development (OT-DSD) locally has been to remove ovotestes and all gonadal tissue discordant with the assigned gender. This was done at the earliest opportunity, and usually preceded superficial cosmetic procedures allowing the child to fit the assigned gender role.

This policy falls within the treatment modalities noted in the literature for patients with OT-DSD, where the range is from early complete excision of gonadal tissue together with all necessary cosmetic procedures, to a conservative approach of post-pubertal excision of discordant gonads and late cosmetic procedures.[1,2,3]

The aim of this chapter was to look at the accumulated data and assess if our management choice is valid.

PATIENTS AND METHOD

This was a retrospective study of 111 patients treated for OT-DSD over a 23-year period (1984-2006) at the Department of Paediatric Surgery, University of Natal, Durban, South AfricThe investigations looked at the clinical presentations of patients, i.e. gender phenotype, gonadal function and histology. In addition the assessment looked at the choice of assigned gender, and patient follow-up.

RESULTS

External genital appearance

The external genital appearance had a considerable genital spectrum. The most female form, the Prader classification 1, was seen in nine patients.[4] Eight patients had a separate urethra, vaginal and anal openings, and on a very superficial inspection nine patients looked female with a clitoris or micro penis and labia. The remaining patients all had a penile structure ranging from small for age to two patients with a large for age penis. On the 'male' side of the genital spectrum, the Prader classification 5, there were five patients who had a scrotum and a penile urethral opening.

Gonads were palpable in 53 patients. Fifteen of these patients had bilaterally palpable gonads and in 38 there was a single palpable gonad in an inguino-scrotal position.

Secondary Sexual characteristics

There were only three children who presented in their teens with spontaneous secondary sexual features. They were a ten, 11 and 13-years old at the time of their presentation. All had bilateral breast development without the aid of hormone supplementation. None had any features of menses.

Serological evaluations

Steroid assays were done in 47 patients with OT-DSD, the range of investigation was incomplete in many patients. The steroids assessed, the number of patients assessed, range of values and mean levels are shown in Table 1.

Table 1. Showing steroid assay, number of patients and values[5]

Steroid assayed	Normal range (2-24 Months)	Number tested	Low range	High range	Mean
Cortisol	83-690 mmol/l	46	21.03	1195	294 mmol/l
Oestradiol	< 37 pmol/l	23	31.9	263	71.2 pmol/l
Testosterone	0.2-2.98 nmol/l	36	0.1	10.8	2 nmol/l
Progesterone-17OH	0.2-9 nmol/l	34	0.1	37.0	6 nmol/l

Testosterone levels were tested in 36 patients. The age of the patient when the test was done, how many patients tested and the result is shown in Table 2. The "3-day Basal Testosterone" test following 5ß-HCG stimulation were done in 10 patients, of whom six had a good and four a poor response (Table 2). Of the six patients who had a response showing an increase in level to a mean 6.8 nmol/l (2.7-12.8 nmol/l) or 7-times increase in concentration, five were children <7 months of age and one was 13 years. The other

Table 2. Results of Testosterone assays in 36 children

<table>
<tr><th>Assay</th><th>Age</th><th>Patient No.</th><th>Result</th></tr>
<tr><td rowspan="4">Testosterone</td><td rowspan="2">< 6Mths</td><td rowspan="2">15</td><td>13 Normal</td></tr>
<tr><td>2 Low</td></tr>
<tr><td rowspan="2">> 6Mths</td><td rowspan="2">21</td><td>3 Normal</td></tr>
<tr><td>18 Low</td></tr>
<tr><td rowspan="4">5 ß-HCG stimulation test</td><td rowspan="2">< 6Mths</td><td rowspan="2">6</td><td>5 Normal</td></tr>
<tr><td>1 Low</td></tr>
<tr><td rowspan="2">> 6Mths</td><td rowspan="2">4</td><td>1 Normal</td></tr>
<tr><td>3 Low</td></tr>
</table>

four patients who had a poor response to the ß-HCG stimulation, had an increase of less than 0.78 nmol/l (range 0.70-1.00 nmol/l), three patients were older than one-and-a-half years.

Internal genital features

The internal genital features showed a range in the presence of Müllerian and Wolffian tubular structures, as could be seen in chapter 4. There were 57 patients with both vagina and uterine structures and 15 without any remnant of a Müllerian structure at all.

The internal inspection also showed there were 94 patients with pelvic gonads, in 37 patients there was a single intra-pelvic gonad. In the remaining 57 patients bilateral gonads were found within the pelvis.

Gonadal histology

Gonadal histology showed that of the 217 gonads, there were 118 ovotestes, constituting 54% of all gonads and the majority (89%) were of a mixed ovotesticular type. The remaining gonads were 59 ovaries and 40 testes.

Gender assignment

The overwhelming majority of the 111 patients already had a gender assigned by the parents prior to admission. On the admission registration, when asked what the gender of the child was, 60 patients were thought to be males and 51 females. Twelve patients changed gender, three from female to male and nine from male to female gender. Seven of the nine children who were admitted as males, were less than one year of age on admission and had an administrative gender change within the first year of life (range 1 week- 9 months), shortly after investigations were complete. Six of these children had an ovary, which was retained. The two remaining children who had a male to female gender change, were 1.5 and 2-years of age on admission, displayed female behavior by six and eight years of age, and also still had an ovary. Of those three children who changed from female to male, one was 1.5-years old when the father insisted he wanted a son and the gender was changed accordingly. The other two patients changed at seven and eight years of age because they expressed their male gender and displayed strong male behavior. Only one had an ovary, which was excised after the time of gender change.

Follow-up visits

The records of the post-investigative clinic visits showed that 11 (10%) of 111 patients failed to return for a single follow-up visit following diagnosis. The remaining 100 patients came for reviews within the first 6- 12 months. The first two visits usually occur within the first 6-months following investigations. The numbers of subsequent follow-up are shown in Table 3, together with the patients 'hospital of origin i.e. peripheral or local. The

Table 3. Record of number of patients seen at follow-up visits

Years post-investigation	0	2	4	6	8	10	12	13
Local	11	9	4	2	4	0	0	2
Peripheral	14	13	11	11	7	5	4	3

oldest patient at follow-up was 13 years of age who was on hormone supplementation. Patients >13-years of age are followed by the adult endocrine/gynaecologist.

Gonadal surgery

All patients had gonadal biopsies, representing 217 gonadal histological assessments. Fifty-nine patients had 20 gonads excised, but the results were not traceable / incomplete. The remaining 52 patients had a completely traceable histological record, showing that 83 gonads were removed in total, of which there were 74 ovotestes, seven ovaries and two testes. Comparing the biopsy result with the histology of the completely excise gonad showed that 22 biopsies were incorrectly reported. Here 11 testes and eight ovaries on biopsy result should have been reported as ovotestes when more gonadal tissue became available with the whole gonad. The mean age at gonadectomy of the children was 7-months after the primary admission (range = 1-week to 8-years).

DISCUSSION

Despite the improved explanation and communication regarding this condition to the parents, and greatly improved transport network in the area, our records still showed that 11(10%) patients never returned for a single review after a diagnosis was made. The remaining 100 patients were all seen in the subsequent year, but this was followed by a steady decline in patient numbers over the months and years. The significance of patients failing to return is that they are left with both their ovarian and testicular gonadal tissue *in-situ*, and have not had any surgery to help them fit into the chosen gender. For those patients who abscond once the gonadal tissue has been removed, puberty was not induced. Both these conditions are of concern.

The majority of patients (n=99) maintained their assigned gender. Of the 12(11%) of children who changed gender nine patients did so following investigative work-up. The remaining three patient changed gender due to compelling behavioural changes that the child displayed. The significance is that for the majority of patients the chosen gender based on phenotype or other clinical parameters were correct, but for that minority of patients where an incorrect gender is chosen at the beginning, allowances must be made for a change of gender by leaving potentially functional gonads and gender structures.

The gonadal function was significantly different between ovaries and testes. The ovarian tissue showed a normal prepubertal oestrodiol production. This was in keeping with reports, which show individual ovaries would result in the development of secondary female characteristics.[3] The clinically functioning ovaries could be verified in only three of our patients, who presented with breast development. Two patients had a remaining ovary and the third patient still had an ovotestis. One patient was reported to be sexually active.

The testicular hormonal profile results show a time related deteriorating testicular function, to the extent that after 6-months of age, the hormonal function of the OT-DSD testes is abnormally low and deteriorating. These results are comparable to the results from other units.[6]

Ovotestes were found to be the commonest gonadal type in the patient with OT-DSD.[3,6,7] Of these ovotestes 85% consisted of a variable mixture of ovarian and testicular tissue.[8] This made the division of this structure into ovarian and testicular components impossible. The development of malignancy in the gonads of DSD patients is very low, and tumours are reported exclusively in both gonadal types *in-situ* of patients with OT-DSD.[9,10]

In view of the above, a policy of gonadal management should include the following. Retention of ovaries until a final decision can be made on the gender of the child. This would give the child wishing to be a female the advantage of puberty and breast development without the risk of malignancy. Children wishing to be males should have an early excision of testes and ovotestes, thereby removing non-functional and potentially harmful gonadal tissue. Ovaries in children brought up as males should be retained until later, despite the risk of secondary genital features e.g. clitoral enlargement in females or breast development and per-urethral bleeding in males, should the child fail to return for follow-up.

Despite the risk of leaving inappropriate gonadal tissue in patients who may abscond from follow-up, removing all gonadal tissue would render a percentage of these children agonadal. Leaving prepubertal children agonadal is of concern in the face possible loss to follow-up, and would also deny some the chance of developing puberty spontaneously.[1] The misdiagnoses of 22 gonads is more than reported in the original study of fewer patients, and where the majority of histological misdiagnoses were testes.

CONCLUSION

The previous recommendation of early excision of ovotestes and gonadectomy of all discordant gonads to the assumed gender requires changing in the present circumstances. Ovotestes and testes still require early excision in children of both genders, and ovaries need excision only when the child is a male after the 7-8 year of age.

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Chapter 9

Management of the African child with OvoTesticular Disorder of Sex Development

This chapter is based on Wiersma R. Management of the African child with true hermaphroditism.

***J Pediatr Surg.* 2001; 36(2):397-399.**

ABSTRACT

A disproportionate high number of patients with Ovotesticular Disorder of Sex Development (OT-DSD) are seen among the South African black people. These patients constituted 51% of all children in a local study of Disorders of Sex Development.[1]

The management of patients with OT-DSD was complicated by the lack of a clinically determinable gender and accordingly a protocol for the management of these patients was established in 1996.

The aim of this review was to establish if the protocol for the management of children with OT-DSD in Southern Africa is effective.

INTRODUCTION

A disproportionatly high number of patients with OT-DSD has been reported among the South African black people, the cause of which has not yet been elucidated.[2,3] The incidence was reported to be as high as 51% of all paediatric patients seen with DSD in a local study, and confirmed recently by re-examining our cumulative data.[1]

Unlike other forms of DSD, children with OT-DSD do not have a clinically determinable gender. The eventual gender of patients is determined more by the exposure of the foetal brain to androgens, then by the karyotype, type of gonad or phenotype.[4,5]

The age a child develops an awareness of gender is between 2-3 years of age, and they are able to distinguish between the sexes by 3-4 years of age. By 6 years children spend more time with their own sex, such that by the time they go to preschool they already have a sense of body sex and will have developed gender identity constancy. Children with DSD however show a signs of gender confusion from 2-4 years of age.[6,7] Local experience has shown that children with OT-DSD were able to determine their own gender between the ages of 6-8years, even if brought up in the 'wrong' gender.

On the basis of the clinical examinations, laboratory investigations, and gonadal histology, it was difficult to predict the future gender of children with OT-DSD. The management of patients with OT-DSD in a strongly gender based society has therefore been problematic.

Developing an appropriate method of management for patients with OT-DSD was the topic of a previous study.[1] The purpose of this review was to assess whether the management protocol, which relies on the child's awareness of its own gender by 6-8 years of age, is effective.

The importance of a multidisciplinary approach in the full management of all patients with DSD cannot be over emphasized. The long-term input from paediatricians, psychologists, endocrinologists, surgeons, social workers etc. are vital to the eventual well being of the patient and parents alike. No change should be brought about without a full assessment by all the above role players, where these are available.

MATERIALS AND METHODS

A management protocol was adopted by our department to allow children with OT-DSD to best fit into a society where they can school and socially interact, until the child can assist with determining its future gender. The following management protocol was implemented locally:

Children unaware of their gender (0-6 years of age)

Gender assignment

Where the child has not yet been assigned a gender by the parent, a decision is made on the best suited gender for that child following full investigation and a wide discussion undertaken with all the role players and the parents. If on the other hand the child is already several months of age and has been assigned a gender by the parent, this gender should be maintained, unless the investigations show grounds for change.

Gonadal management

The ovotestes and testes are planned for excision in the first 2 years of life.

Corrective surgery

Minor external cosmetic surgical procedures are planned for children raised as males. This may be done where the parent is concerned about the child's ability to fit into the assumed gender role. Release of the tethered penis and correction of chordee could be done without compromising any future male or female gender cosmetic surgical procedures. For OT-DSD children raised as females, no cosmetic surgical procedures should be planned until the child is certain of her female gender.

Children aware of their gender (6-8 years of age)

Gender assignment

Where the child agrees with the gender of rearing, no administrative changes are necessary. Where the gender of rearing was incorrect, suitable changes need to be implemented. This was usually accompanied with name and document changes, which remain difficult and sensitive issues. Informing relatives, friends and colleagues is extremely difficult, and here psychological assistance for the patient and parents are vital.

Gonadal management

In children where the gonads are discordant to the chosen gender, i.e. ovaries or testes, these require excision. Retention of hormonal active gonadal tissue in keeping with the gender of the child, allows the child to have a normal puberty. The management of those patients who had all gonadal tissue removed involves endocrine specialist care. In children raised as males this may require the stimulation of penile growth using DHT or testosterone injections. Children without any functional gonadal tissue require the induction of puberty at approximately 11-12 years of age. Once growth has stopped and secondary sex characteristics have developed, hormonal treatment needs to be continued life-long.

Corrective surgery

Older children who have decided which gender they are most comfortable with, will require some surgical procedures to allow them to fullfil the chosen gender role. For both genders this may involve extensive reconstructive genital surgery. These procedures are covered in the following chapter.

Looking at the management of all 111 OT-DSD patients followed in this thesis shows the following outcomes.

RESULTS

Eleven of the 111 OT-DSD patients did not return following their initial investigations, they have not received any surgical procedures beyond the diagnostic investigations and are considered lost to follow-up. There were 71 patients who in the time frame of this study, were old enough to be aware of their gender. Forty children were brought up as females and 31 as males. The remaining 29 patients, eight females and 21 males, were too young to recognize what gender they were.

Of the 21 'males' too young to be able to express their gender, nine patients have had a correction of chordee and fusion of the labioscrotal folds to assist in their male upbringing. No further genital surgery is suggested until they are able to express their gender. Thirty-one males were old enough and confident of their male gender. Here 22 have had the initial chordee and scrotoplasty where needed, and have since had a urethroplasty. Two further patients are on the waiting list for a urethroplasty.

There were 47 patients raised as 'females' who returned for a follow-up. Altogether 36 patients had a gonadectomy. There were 30 patients who were old enough to know their gender and had successful clitoro- and labioplasties. Only one 13-year old child is unsure about her gender, but the remaining patients were all confidently female. In 16 patients vaginoplasties were done between 8 -15 years, some by the gynaecologists. Two children were 10 and 13-years of age at their last review had an external opening vagina, and three children have had vaginoplasty and still required dilatation. Three patients have puberty induced breast development.

Parents had already chosen the gender in 93(84%) children at the time of referral. The gender originally chosen for the child was generally adhered to into the teenage years. Twelve children however did change their originally chosen gender. Three children changed from female to male. In one child where the father insisted on a son instead of a daughter, had the gender changed to male at 14-months of age. Two 'female' children displayed clear male behaviour at the age of seven years, refused to wear dresses or perform female tasks, and only played with other boys. Both changed gender following extensive counselling and are doing well as males. One is now 11-years of age and has all his penile surgery done, but has an empty scrotum. The lack of gonads in the scrotum was initially a problem to parent and subsequently to the child in the early teenage years. The last child still needs his urethroplasty completed.

There were nine children who changed from male to female. Seven children changed gender following the investigations, which suggested a gender change would better suit the child. They changed gender within the first year of life. The last two children were raised as males for six and eight years respectively, then changed to females. Both were well adjusted on later review. One of these females, is now nine years of age, is sure of her gender and awaits a vaginoplasty in a couple of years time.

DISCUSSION

OT-DSD constitutes a small percentage of all the DSD patients seen in most practices worldwide.[2] There are exceptions to this incidence, and in Southern Africa such patients are seen in far greater number.[3] This was confirmed in our own study, in which 51% of all patients investigated for DSD were shown to have OT-DSD.[1]

Large numbers of patients with OT-DSD continue to be seen at our clinics, but follow-up of our patients also continues to be a problem. Eleven patients were lost to follow-up despite improved transport in the region and greater awareness of communication efforts.

The present protocol allows for the gender change at the child's or parent's request. Historically such children were raised as females and they had an early clitoroplasty, which prohibited further gender change.[8] This causes major social and psychological problems.

Early vaginoplasties continue to require dilatations to maintain a sizable orifice.[9,10] One of our patients had a vaginoplasty at six-months of age and required a re-do procedure at 4-years of age to reopen the orifice.

CONCLUSION

Based on the above, the suggested management protocol appears to be suitable for patients with OT-DSD in our environment.

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Chapter 10

Surgery and the patient with OvoTesticular Disorder of Sex Development

ABSTRACT

The care of children with Ovotesticular Disorders of Sex Development (OT-DSD) requires the long-term input from a team of specialists. The surgical team has a substantial role to play in the management of these children.

The Investigative, Diagnostic and Cosmetic surgical procedures required in children with OT-DSD need to cater to each child and discussed here. This chapter examines some of the surgical aspects of these procedures.

INTRODUCTION

The complete management of patients with Ovotesticular Disorders of Sex Development (OT-DSD) requires the long-term input from a team of paediatricians, psychologists, endocrinologists, social workers, surgeons, etc.

The scope of surgical procedures required in patients with OT-DSD is unique among the Disorders of Sex Development (DSD) conditions, particularly as not all DSD patients require surgery. The surgical procedures may be categorized into the following;

Investigative & diagnostic surgical interventions, consisting of an examination of the external and internal genitalia together with gonadal biopsies should be done as soon as possible. This will establish a diagnosis and lead to a gender assignment of the patient based on the most suitable gender of rearing for that child.

Management of the gonads may be necessary in patients with OT-DSD, as both ovarian and testicular gonadal tissues are present. Although gonadal tissue in these patients are regarded as infertile, ovaries have normal hormonogenic properties and are able to induce normal female puberty, but testes lose their hormonogenic ability during the first year of life.[1] Retention of both gonadal tissue types may lead to the development of unwanted feminizing features in children raised as males, and the risk of malignancy of the gonads, especially ovotestes in patients with a 46,XY karyotype.[2,3] Our management policy on gonadal surgery is discussed in chapter 9.

Gender and Cosmetic surgery_may be necessary to allow the young child with OT-DSD to give the appearance of the assigned gender.[4] This may be divided into:

- o *Early interventional surgery*. Great pressure is brought on the surgical team to make patients, who are not yet able to express their innate gender, fit the gender assigned to them. Our policy for this early surgical management is discussed in Chapter 9.
- o *Gender surgical procedures*. This is for older children or young adults, who are able to express their own gender. The gender establishing surgical procedures, i.e. clitoro- and urethroplasties, assist with giving these patients the male or female gender confidence.[5,6] Subsequent corrective cosmetic procedures, including insertion of artificial testes and breast augmentation, are done at the appropriate time in the patient's life.

This chapter discusses our experience with the gender surgical procedures. The decision to change the external genital appearance of a person should only be made following wide consultation with all the above mentioned role players. Although this must include the parent, the child's awareness and understanding of his or her gender is the most important driving force for this change, despite being a legal minor.

GENDER AND COSMETIC SURGERY

There is a large spectrum of management options for the care of the OT-DSD patient. This ranges from the removal of gonadal tissue and changing all DSD children into females to selective surgery befitting the child's suggested gender tendency.[7,8,9] The most frequent form of surgery involves the external genitalia and in particular the enlarged clitoris or tethered penis.

Clitoro- and Labioplasties

97% of our patients with OT-DSD have an associated enlargement of the foetal phallus. Where the child was raised as a female, a clitoroplasty in conjunction with a labioplasty was one of the commoner procedures required. Parental pressure to do this procedure early should be tempered with caution, and await the child's ability to express the innate gender at about 7-8 years of age.

The reason for the combination of the clitoroplasty and labioplasty is that with the enlargement of the foetal phallus, the tissues that should have developed the labia minora have become stretched and incorporated in the penile shaft. Reduction of the penile size to a clitoris allows the excess skin to be mobilized and given the labia minora appearance.

Many surgical procedures for clitoroplasty have been described, however all have the real risk of a reducing the sensation of the glandular structure as a result of damage to the glans clitoris nerve supply.[10,11]

Of those children raised as female, 36 have to date had clitoro- and labioplasties. In the early years of this review, occlusive sutures of the corpora cavernosa from the pubic rami distal to the glans penis were done. This procedure consisted of serial ligatures along the corpora cavernosa within the Buck's fascia sparing the dorsal neurovascular bundle. This collapsed the corporal structures, which were then sutured onto the pubic midline to help fill up the mons pubis. The glans in most patients required to be reduced in size by excision of lower borders. In the latter part of this study the ventral halve of the penile corpora cavernosa and glans were excised and the remaining structure was folded onto itself and closed. There are several procedures that describe this.[12,13]

Urethroplasty

The presence of a penis in patients with OT-DSD is usually associated with a posterior hypospadic anomaly, often scrotal, but many perineal hypospadias. The surgical correction for this may be done as either a single or staged procedure.[14]

Where the glans penis is tethered directly or by a short urethral plate to the scrotum or perineum, the first stage of reconstruction is an orthoplasty (correction of the chordee). Here the ventral release of the glans leaves a short urethral plate in the perineum. The release of the penile shaft skin, the excision of a fibrotic fascia and skin closure using a series of 'z'-plasties, allows the penis to become straight. Such a penis will however be totally devoid of corpus spongiosum and a urethral plate. No further surgical procedures should be planned for the next 6-month to a year to allow the tissues to re-establish vascularization and the penis to grow straight.

Thirty-one patients raised as males had a correction of chordee as a first procedure. These patients had normal to small for age penises, but all had a hypospadias with tethering of the penis to the scrotum.

The Duckett 'transverse island flap' urethroplasty using the prepucial mucosa without hair follicles and few sweat gland has been the procedure of choice to reconstruction of the neo-urethral tube along the length of the ventral penis.[14] Fourteen such 'transverse island flap' procedures have been done where there has been insufficient urethral length. In three patients where the urethral plate was longer, the 'tubularised incised plate' (TIP) urethroplasties have been done.[15] Urethrocutaneous fistula, dribbling urine and poor stream are the main complications seen locally. Other surgical procedures, using bladder or buccal cavity mucosa have also been described, but have not been used locally.[16]

In those patients where the penis is not of normal for age size, stimulation of growth should be attempted by the paediatric endocrinologists. Locally injections of 50mg IM testosterone monthly x three months have been used with good effects. This procedure has been noted to give a 53% growth rate in the first month of application. There is a 27% increase on the second month of application prior to puberty.[17] No growth has been noted in post pubertal patients. Eight patients have had testosterone injections before urethroplasty and three patients have had post-urethroplasty injections. The numbers are too small to compare results. The application of Dihydrotesterone cream has also been described with similar effect.[18]

Vaginoplasty

Vaginoplasty is the procedure where a vagina is given an opening onto the fouchett or perineum. This procedure may be divided into two types. The first type is where there is a sufficiently large natural vagina that can be brought out. The second type is where the vagina is too small or where no vagina exists and a neovagina needs to be made. The timing and type of vaginoplasty is still under discussion due to the complication of vaginal orifice stricture.[11]

In the first type there is a natural vagina, which joins the urethra to form the external opening as a urogenital sinus. Here the further management is dependent both the distance between the perineum and the vagina proper, as well as the length of urethra from the urogenital sinus to bladder neck. Where the urogenital sinus is short, and the separate urethra and vaginal lengths are adequate, then an early vaginoplasty may be done with good results.[11,19] However if the gap from vaginal orifice to the perineum is long, leaving a short urethra to bladder neck, then it is suggested that the vaginoplasty procedure is left until the child is of a pubertal age. Here a 'Pull-through' vaginoplasty may be required, leaving the urogenital sinus as the urethra and mobilizing skin flaps or using an intestinal interposition to fashion a vagina. Urinary continence and vaginal orifice stenosis are frequent complications. The 'total urogenital mobilization' has been described to prevent some of these complications, but there are no long term studies.[20]

Of the children in our study raised as female, 16 children were old enough to decide they were female and had vaginoplasties done. In all these patients the urogenital sinus was short and a vagina could be brought out to the fouchett or perineum. Such vaginoplasties may require dilatations, which are best left until after puberty.[11] Firstly the dilatations are done to keep the perineal opening patent, and secondly to enlarge the vaginal calibre.[21] Certainly such dilatation can achieve enlargement of structures from 5mm diameter to a size that may generously accept a tampon or allow intercourse without pain. Vaginas that are present should be maintained and if necessary augmented with other tissues along its length, e.g. Ileum.[21,22,23]

The second type of vaginoplasty is the construction of a neo-vagina, leaving the urogenital sinus as the urethra. The choice of materials for the neovagina is varied, i.e. skin, short bowel, colon or amnion. There are no perfect substitutes and all have their detractors. Here the operation is also left until the child is pubertal, for reasons that early surgery usually leads to stricturing of the orifice. If the operation is done at the time of commencement of sexual intercourse, then there is a process of natural dilatation.[24,25]

Our record only shows two such neovaginoplasties, but as these patients were too old for the paediatric surgical clinic these records would not be a true reflection.

Subsequent surgical management

Insertion of artificial testes

This should only be done when the patient is of pubertal age. These silicone containing structures of adult sized testes are sutured on the median wall of the scrotum to ensure a normal appearance with some fixity.

Breast reduction or augmentation surgery

In the older child the breast or lack there of may require surgery. Mastectomies may be required in children raised as males, but where the ovarian tissue has already had some effect on the breast tissue. Here a subcutaneous mastectomy removing all the glandular breast tissue, but leaving the nipple-areola complex is the treatment of choice.[25]

The outcome of these surgical procedures is decided on;

- o Cosmetic appearance

- o Anatomical outcome (Vagina size, patency, Penile size)
- o Revision rate
- o Complications
- o Psychological quality of life
- o Sexual function
- o Patient satisfaction. (most important!)

CONCLUSION

Timing of cosmetic surgical procedures is crucial to good outcome. The best surgical results are seen following wide consultation and patient participation in the final gender decision.

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Chapter 11

Disorder of Sex Development conditions in children and adolescents: surgical, ethical and legal considerations

Maharaj NR, Dhai A, Wiersma R, Moodley J. Intersex conditions in children and adolescents: surgical, ethical and legal considerations.

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ABSTRACT

Approximately one in 2000 children globally is born with a Disorder of Sex Development (DSD) condition. There is unfortunately a relative paucity of data on the choices and the surgical and psychosocial outcomes in patients who undergo genital surgery for DSD conditions and ambiguous genitalia, especially in developing countries. Specialists in these and other countries, where patient follow-up is generally poor, are faced with the daunting task of offering the appropriate medical and surgical management, in the absence of guidelines or recommendations.

A surgical procedure in these patients sometimes involves clitoral recession, reduction, vaginoplasty, and gonadectomy. The best surgical outcome is likely to be achieved with a multidisciplinary surgical team; however, the choice of surgery and appropriate timing remains controversial. Some authors have suggested delaying surgery until the child becomes competent to make his/her own decisions.

All procedures should conform to an ethical code of practice and be in the interest of the child. Exhaustive counseling of all parties and informed consent is of paramount importance, as is adherence to laws that protect the rights of the child as outlined in respective constitutions.

Recommendations in this article, which have been put together from the combined input of three departments, are broad-based. They emphasize the need for extensive counseling, informed consent, adherence to ethical and legal norms, a multidisciplinary input and a shift away from a paternalistic approach.

INTRODUCTION

The management of DSD conditions frequently poses a challenge to gynecologists and pediatric surgeons in many countries. These conditions can broadly be defined as imperfect sexual differentiation into male or female.[1] Although surgery for DSD conditions and ambiguous genitalia has been well documented in medical literature, there has, until recently, been a relative paucity of data in both scholarly and lay media on the long-term outcome of affected individuals who undergo 'corrective' genitoplasty. The difficult social and personal adjustments faced by those who undergo gender re-assignment surgery is sometimes highlighted in the media, rather than in medical literature. The following compelling news story is one such example.[2]

"A botched circumcision left David badly mutilated. His parents were then counseled to turn David into a girl. David had to be castrated, have surgical reconstruction, and be given female hormones and psychological conditioning. David became Brenda. The family was not identified in those early years, but David himself finally went public a few years ago. In his book, 'As Nature Made Him,' David revealed that far from enjoying dresses and dolls, he preferred boy's clothes, growing into a confused, rebellious adolescent. David revealed his mother tried to kill herself and he made at least 3 suicide bids before his final successful attempt. A month before his 16th birthday, he began to attempt to rebuild his life, undergoing the first of a series of operations to remove his breasts and create a penis. He later met and married a woman and adopted her 3 children, but the legacy haunted the family. David became morose. He lost his job and separated from his wife. David committed suicide in Winnipeg, Canada, where he had grown up." [Adapted with permission from the Daily News, courtesy Daily Mail].

Unlike in developing countries, the medical profession in Westernized countries is confronted by the critical voices of DSD and feminist consumer groups, further compelling doctors to conform to an ethical code of practice when embarking on surgery for ambiguous genitalia.[3] However, while considering the various entities that define human sexuality, many specialists are still more likely to make a decision on the choice of gender reassignment based on the predominant appearance of the external genitalia and the ease with which successful surgery can be performed. To this end, it is probable that most surgeons are more likely to opt for feminizing genitoplasty and female sex of rearing. In the USA and most western European societies, a female sex of rearing is the more likely clinical recommendation to parents.[4] However, in Africa, particularly South Africa, where a disproportionately high incidence of true hermaphrodites is seen among the South African black population, these recommendations may differ.[5]

SURGICAL PROCEDURES

The two essential elements of feminizing genitoplasty are clitoral reduction / recession and vaginoplasty.[6]

Clitoral reduction/recession

With greater acknowledgment of the vital role of the clitoris in female sexual function, clitorectomy, the removal of both the corpora and the glans, is no longer undertaken in the UK.[4] While the operation of clitoral shaft resection with preservation of the glans on its neurovascular bundle seems logical, and is probably an advance on total clitorectomy or clitoral recession, there is no evidence that the retained glans functions well in sexual/orgasmic terms.[6] Sexual function could actually be compromised by clitoral surgery, with higher rates of non-sensuality and inability to achieve orgasm.[4]

Vaginoplasty

The ease with which *vaginoplasty* can be performed is related largely to the length of the common urogenital sinus. Pena et al have emphasized the appreciation of the intimate relations between the rectum and urinary tract, total urogenital mobilization, and an appreciation of associated Mullerian anomalies for improved surgical outcome.[7] Surgery can be performed early in life, but revision at puberty should be anticipated in some cases.[8,9] The few long-term studies currently available suggest that the majority of girls will require some, and often major, revisional surgery for vaginal or introital stenosis in adolescence.[9,10] Since there is no obvious benefit for vaginoplasty in the very young girl, it seems a feasible option to delay it, until evidence from future research shows benefit. For later vaginal lengthening, various methods of self dilatation are available, and vaginal dilatation with acrylic moulds results in good outcome.[11] For the replacement of a completely absent vagina, colovaginoplasty has been reported with good results by some authors.[12]

The timing of gonadectomy remains controversial. Arguments that cite the potential for malignant change as reason for early gonadectomy are sometimes counterbalanced by the possibility of better bone maturation and body development in the presence of endogenous sex steroids. There are three possible options: (1) early gonadectomy, particularly if they are contained in the hernial sac, or if there are parental concerns over malignant change, or difficulty in accepting female phenotype while testicular tissue is present; (2) late gonadectomy performed as soon as puberty has been completed; (3) no gonadectomy at all in patients who are as well informed as possible about the risks of malignant change. Follow-up of such individuals would need to be assiduous and long term.[6]

In the undervirilized genetic male, follow-up studies by Reilly and Woodhouse on adult males with micropenis (dorsal penile length at least 2.5 standard deviations smaller than mean penis size) have shown surprisingly good outcomes in terms of sexual function.[13] Accordingly, whether the assignment of such individuals to the female gender by surgery should be done needs thorough consideration and therefore should only be undertaken with considerable caution and following full multidisciplinary investigation and counseling, with due consideration given to the sex of rearing where appropriate.

DEFINING SEX AND SEXUALITY

Arguably, an emphasis on feminizing genitoplasty alone might fall short in considering a holistic definition of gender, inclusive of rearing, psychological and social concepts of human sexuality. Debates on this issue are shaped by the continuing issue of whether sexual identity is a biological phenomenon, determined by the genes and the anatomy, or whether it is constructed in society or culture. The former adopts a more 'essentialist' stance, and views sexuality as given by nature and therefore fixed and unalterable. The latter sees sexuality being organized through the regulative discourses of modern societies. This view will naturally support reconstructions and re-inventions of sexuality and explore the questions posed by those living with DSD conditions about the existence of a third kind of gender, i.e. understanding subjects in terms of personal preference and self determination, and not simply defining gender as genital function on the basis of the ability to have sexual intercourse, a Freudian concept.

The birth of a universal prescription for DSD surgery is therefore unlikely, leaving gynecologists and surgeons without sustainable guidelines. Feminizing genitoplasty still remains a common management for DSD infants in the developed world because of the clinicians' beliefs that it improves psychological outcomes.[4] In most other countries, not much is known about choice of surgery and psychological outcome.

ETHICAL AND LEGAL CONSIDERATIONS

Of central importance is to take cognizance of the many ethical dilemmas and cultural norms present in societies and the controversy surrounding DSD surgery. First, the psychological issues surrounding sexuality in these patients are inadequately researched and poorly understood.[8] Second, there is no guarantee that adult gender identity will develop as assigned, and finally, sexual function could be compromised by clitoral surgery.[2,4]

Furthermore, the timing of surgery becomes an issue that places considerable pressure on the parents, a significant number of whom may not be adequately informed. Decisions taken by parents for surgery in early childhood would not necessarily be an appropriate reflection of the choice of the affected child. The alternative of leaving the genitalia unaltered, might predispose the child to various difficulties, including difficulties with body image, gender development, and sexual identity. Some authors have claimed, backed by patient support groups, that surgery is mutilating and, as it is essentially cosmetic, it should be deferred until the fully informed consent of the patient can be obtained, that is, when the child becomes competent to make his/her own decisions.[14]

It is therefore understandable why the surgical management of ambiguous genitalia in DSD conditions cannot be assigned a policy, as this would be too prescriptive.

Management must be individualized to specific circumstances. Decisions must be taken in the context of the rights of the child as outlined in the constitution of the country, and must be in the best interests of the child. In South Africa, for example, this is contained in Section 28 of The Bill of Rights of the South African Constitution.

RECOMMENDATIONS

Of primary importance is an understanding of the physical and psychological dilemmas that face these individuals in the future. Informed consent with complete disclosure of all risks, complications, follow-up and potential for impaired sexual function must be provided to parents of children with ambiguous genitalia. Parents must be able to access as much information as possible from all relevant role players and beyond, and if possible, referred to support groups for further information. Counseling and psychological support must be provided to parents in a non-directed fashion and with an open-door approach. Informed consent will also include an awareness of the possibility of non-operative management with psychological support for the child and family.[3] It therefore becomes necessary to refer these individuals to a center that offers a multidisciplinary team. Ideally, this team should consist of a pediatric surgeon, a pediatric endocrinologist, a gynecologist, a biochemist, neonatalogist, psychologist, and social worker. These multidisciplinary teams are a necessary resource for training, research and follow-up, even in economically challenged African countries. However, if these centers are not available, then one should refrain from any potentially harmful practice and postpone surgery until the onset of puberty, or until the child is old enough to make his/her own decision.

The enrollment of affected persons in anatomical, psychosexual, and other aspects of gender research must be in accordance with the various codes and declarations governing ethics of research in human subjects. Unethical research is in conflict with the bill of rights, where present, as contained in a particular constitution.

Finally, a shift away from the traditional paternalistic decision-making role played by doctors, to one inclusive of multidisciplinary input, and an honoring of the preferences of parents, will help in making this challenging and complex decision.

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Part 4

General Discussion

THE AIM OF THIS THESIS

The aim of this thesis has been to study the various aspects of Ovotesticular Disorder of Sex Development (OT-DSD), or what used to be called True Hermaphroditism. This has been done to recognize those patients with OT-DSD from among the children with ambiguous genitalia, establish a diagnosis at an early phase of their development and help them cope with problems associated with this condition.

This is not a new subject and early scientific papers were written about the recognition of OT-DSD.[1] The subject was discussed with greater frequency after a landmark paper in 1979 emanating from South Africa. In this paper there was a review of the literature on OT-DSD, noting the 364 patients who had been described in the world since 1899, and concluding that the incidence of OT-DSD in South African was greater than anywhere else.[2]

Work done in the Department of Paediatric Surgery at the University of KwaZulu-Natal, South Africa has shown that the Southern African patients with OT-DSD truly represent an unusual experience in this condition. This study looked at 111 patients with OT-DSD who have been managed in this unit since 1984. Presenting some of this work at the British Association of Paediatric Surgical Congress in 2001 prompted the comment "Congratulations, that is the world's largest series by 100%, and I think it is a tremendous experience"[3]

One can therefore ask what this series of papers has produced that is new on the topic and what might be worth noting. Of equal importance is noting what the remaining gaps in our knowledge on this subject are.

NEW OR HIGHLIGHTED INFORMATION

The Investigative protocol

Making a diagnosis of OT-DSD requires a high index of suspicion as there may only be subtle anomalies of the genitalia, and very few diagnostic pointers. This led to the local establishment of a standardized investigative protocol in 1992. This consisted of a chromosomal assay, examination of the genitalia under anaesthesia and an internal genital assessment with gonadal biopsy. This protocol allows for an expedited diagnosis in all patients, particularly in an environment where OT-DSD is common, and gonadal biopsy is a prerequisite for its diagnosis.

The function of the examination under anaesthesia, urethroscopy and internal genital examination is to demonstrate any abnormality of gender. This would highlight the presence external genitalia not in keeping with the gender of rearing, the presence of any Müllerian structures and abnormal gonads to suggest a Disorder of Sex Development (DSD) condition. This would then indicate that further steps need to be taken to define the cause. Here the chromosomal make-up, steroid chromatogram and gonadal histology will define the type of DSD the patient has. This type of management was new in the 1990's, although the literature now has articles describing similar findings from areas where OT-DSD is not common, but where is felt that an internal inspection of the genitalia and gonads may be diagnostic.[4,5]

The importance of this investigative protocol cannot be over estimated. Too many patients have been treated for suspected DSD conditions based on single investigations such as a steroid chromatogram, only to be later diagnosed as OT-DSD when this protocol was instituted. A change of diagnosis at that late juncture meant that the opportunity of an early combined gender assignment was missed, leading to parental anguish and embarrassment regarding the child's gender.

This protocol will aid the medical team and psychological services to prepare the parent and child for what lies ahead. Early investigation and diagnosis allows for easier acceptance for gender change, if this is needed.

The use of laparoscopy in the internal examination

The inspection of the internal genitalia together with the gonadal biopsies is a crucial part of the investigative protocol. Since the advent of laparoscopic surgery, its use in the examination of children was a new innovation in the 1990's, but was adapted to our children with ambiguous genitalia and has been the only method of internal genital examination and gonadal biopsy since 1998. It has since been well described and shown to be better than an open laparotomy in the literature and in Chapter 7.[6,7]

The use of this technique is now also described in children and neonates, even where OT-DSD is not likely to be a major concern.[5,7] The aspects of the internal examination that were improved with the laparoscopic method were the vision of the internal genitalia, the appearance and assessment of the gonadal position. Laparoscopy is a far less invasive procedure, allowing the patient to be discharged the same day and leaving a cosmetically smaller scar in the patient.[4]

Not discussed in the literature on laparoscopic surgery is the manner in which gonadal biopsies are taken. The method of gonadal biopsy, where by the gonads are grasped with tissue forceps and gently delivered into the 5mm lateral ports under vision and then the peritoneum is deflated and the port with gonad delivered outside the abdomen. This method was developed locally and allows the gonads to be safely biopsied in the suggested manner outside the abdomen, as described in Chapter 7. This method makes the biopsy a quick, easy and safe procedure, which allows for a more representative biopsy sample to be taken.

The gonadal histological pattern of the OT-DSD

In OT-DSD the pathognomonic feature is the presence of testicular tissue with seminiferous tubules and ovarian tissue with follicles in the same patient. The commonest gonad found in these patients was the ovotestis, which is a combination of these two gender opposite tissues.

In the literature on OT-DSD there are numerous descriptions of ovotestes.[2,8,9,10,11] Whether these descriptions originated from the Western Cape in South Africa or from Europe, the ovotestes are described as bipolar structures**.** Taking biopsy samples from such bipolar structures requires a snip from both gonadal poles and one knows exactly whether the gonad is an ovotestis, testis or ovary. In view of this bipolar arrangement, the management advice has been to remove the portion of gonadal tissue discordant to the assigned gender.[10] It was interesting that the original descriptions of the ovotestes were exclusively of bipolar structures and in the discussion it was asked why the distribution of ovarian and testicular tissue in the ovotestis was not more random**.**[8] There was one description of a mixed ovotestis, but there too it was noted that the common ovotestis was bipolar.[9]

When biopies were taken from the gonads in our patients, we found that 22 of the gonadal biopsy samples, were in fact found to be ovotestes when the whole gonad was subsequently analysed. As a result, ovotestes were found to be more common than originally thought. What was also found was that 89% of the local ovotestes consisted of two mixed types. Firstly the <u>admixed</u> type (44%), consisting of an outer mantle of ovarian tissue of variable thickness, surrounding a central core of stroma, containing scattered foci of ovarian and testicular tissue of different sizes. Secondly the <u>compartmentalized</u> type (56%), consisted of an ovary, which in the lower portion encapsulates a variable sized core of testicular tissue, as shown in Chapter 6. The third type of ovotestis was the bipolar type (11%), with a variable interdigitated junction of the two tissue.[12]

The relevance of these findings is two-fold. Firstly in obtaining a representative histological sample, a different method of biopsy had to be developed from that used for the bipolar goadal structures. A large enough sample had to be taken to give some representivity of the histological picture, yet leaving enough gonadal tissue in case this was a normal gonad or an ovary that could be left in-situ to function normally. The pole-to-pole wedge biopsy was developed here to fulfil those criteria.

Secondly, the gonadal histological findings impacted on the management of these patients. In the local setting one cannot leave the ovotestis *in-situ*, because it cannot be cleaved into its composite parts. Therefore unlike the practice elsewhere, conservative management is not possible here and the whole ovotestis needs to be excised**.**[10,13]

The management of patients with OT-DSD

It is generally agreed that the investigation of children with ambiguous genitalia should commence at the earliest opportunity to establish the most appropriate gender for the child with DSD and outline the management policy for that condition.[14] Once the diagnosis of OT-DSD has been made, some diversity of opinion develops on how best to manage such patients. This is partially dependent on the patient's age at presentation, but also on the type of gonad that these patients have.

The assignment of the gender for a child has given rise to some differences of opinion. These range from raising all patients with a vagina or a micropenis as female, to a more conservative wait and see approach.[2,13,14,15] In patients with OT-DSD the gender that the child is likely to assume at 6-8 years of age is unknown.

The policy adopted here follows the review of many patients with OT-DSD. Children under 7-8 years of age who present to the clinic are investigated and have consultations with the psychologist. The most appropriate gender is selected based on the predominant genital and gonadal features. If this child is older than one year of age and a gender has already been assigned by the family, then that gender should be maintained. At this time inappropriate gonads should be removed, but no cosmetic procedures should be planned. The parents must be made aware that the child may wish to change the gender at a later stage.

If the child is older than eight years of age, following medical and psychological consultation, the gender elected by the child is adopted. Following a period of orientation, all ovotestes and discordant gonads are removed and the necessary surgical procedures are done to assist the child to assume that gender. Vaginoplasties for those children raised as female without a perineal opening to that structure, are controversial and tend to complicate (see Chapter 10). Locally these procedures are left until the female child approaches puberty, unless the urogenital sinus is very short.

DATA MISSING FROM THIS THESIS AND THE SUBJECT IN GENERAL

Aetiology of OT-DSD

Chapter 1 examined the origin of the normal gender development and gave an explanation of the anomalies that occur in that process. It also highlighted the fact that there is no clear designated cause for OT-DSD, at the moment there are only four likely theories.[16] One of the assumptions is that there are multiple H-Y structural genes on the Y chromosome and that if these are split by translocation to another chromosome, the number of gene copies translocated may determine the dosage of testicular development in the gonadal differentiation.[8] The development of the ovotestis then leads to the secondary changes in genitalia of the patient.

Discussions on the matter of aetiology in 1976 already led to the correct assumption, that OT-DSD is probably not a single cause, but rather a host of related disorders which have in common the involvement of both male and female gonadal tissue.[2]

With genetic analysis becoming more readily available, this is where future investigation of OT-DSD is directed and such papers on the genetics of this condition are seen in all the journals.[17,18] Studies from South Africa on OT-DSD patients had highlighted some of the genetic differences seen in the OT-DSD patients found in South Africa as compared to Europe. This included that the condition was seen more commonly among the black population of this country, whilst rare among the Caucasians, and familial cases are rare in the black OT-DSDs whilst this is common in Europe.[19]

It has been noted that the genetic make-up of OT-DSD patients is not homogeneous. The karyotype in the majority of South Africa OT-DSDs was found to be 46,XX, in Europe there appears to be a high preponderance of Mosaic 46,XX/46,XY carriers and in Japan the 46,XY karyotype predominates.[9,20] We can only conclude that as there are very few familial cases and our patients come from diverse locations in this country, one would expect a sporadic genetic mutation as the likely cause and large numbers would therefore be needed to find such a lesion.

The incidence of OT-DSD in the rest of the world is approximately 3%, yet we have repeatedly shown that the incidence locally is very high, 51% is the calculated figure.[3,11] The reason for this will probably come to light when the aetiology of OT-DSD has been found, but at the moment the cause for the local high incidence remains unknown. If the cause of OT-DSD is suggested to be multifactorial, and we are seeing a common end product of genetic mutations occurring in the cascade to sex development, then one must assume that this occurs more frequently here in Southern Africa. In such a scenario we should be seeing other anomalies that occur more frequently as a result of these genetic mutations. This has not yet been found to be the case.

Psycho-social effect of OT-DSD

Some of the psycho-social aspects of OT-DSD have briefly been touched on in Chapters 1 and 11. These included how the gender ambiguity affected the child in terms of future gender identity and fertility. Similarly the effects on the parents of such children were discussed as the child grows up, particularly in a society that has definite gender roles.

Studies of the psycho-social aspects of patients with DSD conditions in general are from time to time found in greater depth in the literature. These are largely long-term reviews of DSD patients from developed countries.[21] Even in these developed nations where the social services and follow-up clinical services are better than here, there is still a significant loss in patients follow-up.

Such studies have shown that DSD is not an easy condition to come to grips with. Patients accept the chosen gender until they themselves realize that the initial gender chosen might be wrong and they wish to change to the opposite gender. This often leads to difficult psychological problems, even under conditions where this is accepted that it may happen. These patients need therapy, as well as assistance, and they themselves need to have the confidence to express that their gender does not suit them.

What has come out of those studies is that later, once the patient is now adult, they may again feel that they are not in the correct gender and may wish to change. This suggests that these patients have great difficulty adjusting to the two gender model and probably have a fringe gender role. Studies of these patients are difficult as they are unwilling to discuss their sexual problems. The problems that have come to light are that DSD patients have a sexual averseness and lack of arousability, which are often misinterpreted as low libido. Some patients avoid intimate relationships and it is important to address fears of rejection. Advice should be on interpersonal relationships not solely on sexual function and action.[22]

Many of the psychosocial effects in relation to the local patients have not really been touched upon. The problems noted above are probably true for our patients, but in the Third World gender roles are more clearly defined and borderline roles less easily tolerated.

What happens to patients long term if left untreated ?

The majority of patients with OT-DSD are recognized at birth as a result of their genital ambiguity, but there is a percentage of patients who are not recognised until late and some never.

Looking at patients who were discussed in other chapters gives us some insight into these patients. Those patients who present late do so because they have secondary sexual development out of keeping with their assumed gender. In the original article highlighting the prevalence of OT-DSD in the South African people, of the three patients presented two had adopted the male gender because they had a penis, although both had hypospadias and chordee.[2] However, the hypospadias was not the reason for presentation, and neither was the abnormal scrotum or the cryptorchidism. The reason these patients sought medical help was for the development of breast tissue. This can be interpreted that privately these patients were able to bear their gender abnormality, either because they did not know different or because they tried to fit-in to a male dominant society. However once the breast tissue developed this was now difficult to hide in a social structure that is strictly gender based, such as is the case in Third World countries. This resulted in the hospital presentation.

One of the long-term effects in such patients recently written about are the malignancies.[23,24,25] Although the incidence of malignancy in patients with OT-DSD are said to be low, long term studies do not exist. Therefore larger studies of 'late' and 'very late' presenters should be done to answer some of the following questions regarding the patient management. What are the long-term effects of leaving ovotestes in-situ, what are the gender choices and the general behaviour patterns of these patients?

Are patients better off without their gonads?

The question of whether a total excision of the gonads in the Southern African OT-DSD is safer than leaving discordant gonadal tissue in such a child remains unanswered, but should form part of future research. In the mean time, leaving children agonadal, such as those children with bilateral ovotestes or with an ovotestis - testis combination, when the likehood of returning for endocrine management in the future is poor, is worrisome.

SUMMARY OF CONTRIBUTIONS

- o Defining a diagnostic protocol.
- o Establishing a method of obtaining a laparoscopic-assisted representative gonadal biopsy.
- o Describing the true histological make-up of the Southern African Ovotestis.
- o Establishing a best practice management protocol for children diagnose with OT-DSD.

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Summary

SUMMARY

This thesis addresses the problem of OvoTesticular Disorder of Sex development (OT-DSD), a congenital condition that affects a person's anatomical as well as mental expression of gender. It is has an unusually high incidence among the black Southern African people. The cause for this frequent occurrence remains unknown.

The diagnosis of genital ambiguity in a child requires the examiner to be suspicious of abnormal genitalia. This recognition should initiate a series of evaluations to establish the diagnosis, suggest an appropriate gender and establish a long-term management plan for that child. Ideally this should commence in the neonatal period, before the child has gone home and a gender has been assigned without due care.

Serving a largely rural and poor population influences the timing and frequency of patient presentation and follow-up. Here among the black African population affected by disorder of sex development (DSD), the importance of the family unit, the local customs, the lack of social services and the fact that hospital follow-ups will be irregular and costly to the family, must be taken into account when management decisions are made.

This thesis is divided into **4 Parts**.

Part 1 examines in some detail the origin of the normal gender development. With this as background, the aberrant causes of gender development and the different DSD types were examined. The causes are numerous, ranging from chromosomal anomalies to end-organ insensitivity, and give rise to a mixed gender picture.

The **first Chapter** looks at the effects of these in-born or acquired errors on the development of gender. The effects of future gender identity and fertility on the child as well as the effects on the parents of such children are discussed.

The **second Chapter** examines the possibility of clinically differentiating between patients presenting with the various causes of gender ambiguity. It made the important distinction that an abnormality of the genitalia did not necessarily imply the child had a DSD condition. Consequently, all children with gender ambiguity should be fully investigated to make a diagnosis of the underlying condition and commence a management plan for the child. This chapter also shows the frequency of DSD types seen locally.

Part 2 consists of five chapters, each looking at some aspects of investigating patients with OT-DSD. How the diagnosis of OT-DSD can be made with some certainty and other features of this condition are covered in the following chapters.

The **third Chapter** looks at the investigations that would be necessary to make a diagnosis of DSD in patients. On the basis of these investigations it was shown that XX-DSD was not the commonest cause of DSD locally. Instead OT-DSD, at 51% of all the DSD conditions, was our commonest cause. It was shown that there were few diagnostic pointers to OT-DSD, there are no 'typical' blood or serum investigations. A standard investigative

protocol was developed to provide an expedited diagnosis of DSD-type, and was found to be relevant to the further management of these patients.

The clinical features of patients with OT-DSD were examined in **Chapter four**, with a view to early recognition. This chapter highlights the range of clinical features and the variability of presentation with age. Untreated these patients adopt their innate gender, but develop problems later with their sexual function or gender roleThe pathognomonic feature of OT-DSD is the presence of ovarian and testicular tissue in the same patient. This is most frequently seen as a single structure called the ovotestis, and these gonads constitute 54% of all the gonads seen in the true hermaphrodite patients. **Chapter five** describes the three distinct ovotesticular types seen locally, which had not previously been mentioned in the literature. These findings are relevant to the further management of OT-DSD patients.

The **sixth Chapter** was a cross-check of the gonadal biopsy method. Here the histology of the biopsy samples were compared to the histological picture of the subsequently excised whole gonads. This chapter shows that gonadal biopsies under-represent the whole gonadal histology, and only the testicular tissue was seen in some ovotestes. The technique developed was the longitudinal, pole-to-pole, wedge biopsy of gonads. This aimed to improve the representation of histological tissue, yet leaving sufficient gonadal tissue to function for hormono-and gametogenesis in patients where such gonads remain, e.g. in children who retain the female gender.

The method of investigating the internal genital organs and obtain representative biopsy specimens was discussed in **Chapter seven.** Here the laparoscopic and open techniques of investigation were compared. The laparoscopic method was found to be the least invasive and gave the better vision of the internal organs and facilitated the gonadal biopsy where necessary.

Part 3 consists of four chapters which complement each other in the discussion on management of patients with OT-DSD.

Chapter eight looks at the functionality of the gonads of patients with OT-DSD and discusses what should be done with the testicular and ovarian tissue found in the same patient. The conclusions drawn were that ovarian tissue may become hormonally active and as such should be left in situ until the gender of the child is fully established. However, testicular tissue loses its hormonal productivity in the first year of life and that together with ovotestes have malignant potential, both should therefore be excised within the first few years of life. Follow-up at the specialist clinic was found to be poor and patients are lost soon after the investigative and primary treatment stage. Based on this lack of long-term care, the risk of malignancy and the small hormonal value of these gonads, it was felt that a policy of removing ovotesticular tissue together with testes if discordant with the gender of rearing could be justified under the present conditionThe early surgical management of patients with OT-DSD is discussed in **Chapter nine**. This chapter suggests that all investigations and the surgical procedures required to allow the child to fit the gender role, without compromising a late gender change, should be done. Early urethroplasties and vaginoplasties have led to unsatisfactory repairs with stenosis, and should be delayed until the gender is fully established. Motivation of parents and patient to return for regular assessment at special DSD clinics and planned late reconstructive surgery was recommended.

The late surgical reconstructive procedures are the topic of discussion in **Chapter ten**. It discusses some of the pros and cons of the procedures and the approximate timing in which they should be implemented.

An understanding of the physical and psychological dilemmas that these patients face in the future is of primary importance in the management of this condition and the topic of discussion in **Chapter eleven**. Informed consent with complete disclosure of all risks and complications must be provided to parents of children with ambiguous genitalia. Parents must be able to access information explained at their level of understanding from all relevant role players and be referred to support groups for further information. Counselling and psychological support must be provided to parents in a non-directed fashion and with an open-door approach. Informed consent will also include an awareness of the possibility of non-operative management with psychological support for the child and famIdeally support to patients and parents should be provided by a multidisciplinary team. Where such facilities are not available, one should refrain from any potentially harmful practice and postpone surgery until the onset of puberty, or until the child is old enough to make his/her own decision. There must be a shift away from the traditional paternalistic decision-making role played by doctors, to one inclusive of multidisciplinary input, and an honouring of the preferences of parents. This will be of great benefit in managing this challenging and complex condition

Part 4 gives an overview of what is known of OT-DSD. It also gives a summary of contributions made by this thesis. These are;

- o Defining a diagnostic protocol.
- o Establishing a method of obtaining a laparoscopically assisted representative gonadal biopsy.
- o Describing the true histological make-up of the Southern African Ovotestis.
- o Establishing a best practice management protocol for children diagnosed with OT-DSD.

This part also highlights what is not known about this condition and where future research should be directed.

Samenvatting

SAMENVATTING

Dit proefschrift gaat over OvoTesticular Disorder of Sex Development (OT-DSD). Dit is een specifieke vorm van een aangeboren afwijkende ontwikkeling van de inwendige en uitwendige geslachtsorganen (genitalia), die zowel de lichamelijke als de geestelijke ontwikkeling van het betreffende kind beinvloedt. De incidentie van deze afwijking is bij de zwarte bevolking van Zuid Afrika ongewoon hoog. Tot nu toe is hiervoor geen oorzaak gevonden.

Na de geboorte van een kind met een onduidelijk geslacht moet zo snel mogelijk een juiste diagnose gesteld worden om verdere verwarring bij de ouders te voorkomen. Hierbij moeten de behandelaars erop bedacht zijn dat niet alleen de uitwendige maar ook de inwendige genitalia afwijkend kunnen zijn. Snel na de geboorte moeten een aantal onderzoeken worden uitgevoerd zodat aan het kind het juiste geslacht (gender) kan worden toegewezen. Vervolgens moet een (lange termijn) behandelplan worden vastgesteld.

De kinderen met OT-DSD die in dit proefschrift worden beschreven komen voor een belangrijk deel uit Durban en wijde omgeving. Door grote reisafstanden, geldgebrek, beperkte sociale voorzieningen en locale gewoonten binnen families komen deze kinderen soms pas op latere leeftijd naar het ziekenhuis, waardoor de diagnose niet direct na de geboorte wordt gesteld.

Dit proefschrift is opgebouwd uit **4 Delen**.

Deel 1 beschrijft de normale ontwikkeling van de genitalia en vervolgens de verschillende vormen van afwijkende geslachtsontwikkeling, ook wel genoemd Disorders of Sex Development (DSD). De oorzaken van DSD kunnen zeer verschillend zijn, voorbeelden hiervan zijn chromosomale afwijkingen of hormonale ongevoeligheid van de zich ontwikkelende uitwendige geslachtsorganen. Dientengevolge zijn de zichtbare afwijkingen aan de uitwendige genitalia zeer verschillend.

Hoofdstuk 1 beschrijft de effecten die veroorzaakt worden door de verstoorde gender ontwikkeling. Wat de consequenties voor het kind en zijn of haar ouders zijn nadat het juiste geslacht is komen vast te staan en de lange termijn effecten hiervan, bijvoorbeeld met betrekking tot latere vruchtbaarheid.

In het **tweede hoofdstuk** worden de verschillende klinische uitingsvormen van kinderen met onduidelijk geslacht besproken. Hierbij wordt benadrukt dat het hebben van afwijkende genitalia lang niet altijd hoeft te betekenen dat er sprake is van een vorm van DSD. Een nauwkeurig en volledig uitgevoerd onderzoek van deze kinderen is noodzakelijk om een juiste diagnose te kunnen stellen, gevolgd door een effectief behandelplan.

Tevens wordt in dit hoofdstuk aandacht besteed aan de frequentie van voorkomen van de verschillende vormen van DSD in Zuid Afrika.

Deel 2 bestaat uit 5 hoofdstukken over belangrijke aspecten van de diagnostiek van OT-DSD. **Hoofdstuk 3** geeft een beschrijving van de verschillende onderzoeken die nodig zijn om tot een juiste diagnose te komen. Op grond van deze onderzoeken wordt vastgesteld dat OT-DSD de meest frequent voorkomende vorm (51%) van DSD is. Alhoewel er geen specifieke bloedchemische of hormonale bloed testen zijn gevonden die van belang waren voor het stellen van de diagnose OT-DSD is toch met behulp van ander onderzoek, zoals chromosomen patroon en inspectie van de inwendige genitalia , een protocol ontwikkeld om tot een snelle diagnose en behandeling van de verschillende vormen van DSD te komen.

De klinische kenmerken van kinderen met OT-DSD worden in **hoofdstuk 4** besproken. De nadruk ligt hier vooral op de vroegtijdige diagnostiek. Er wordt vooral aandacht gegeven aan de verschillende klinische kenmerken van OT-DSD in relatie tot de leeftijd bij diagnose. Opgemerkt wordt dat indien deze kinderen niet behandeld worden en opgroeien in de aangeboren gender, er later problemen kunnen ontstaan omtrent hun sexueel functioneren en gender rol.

Het bepalende kenmerk van OT-DSD is het aanwezig zijn van ovarieel (vrouwelijk) en testiculair (mannelijk) weefsel in de gonade(n) van dezelfde patiënt. Het betreft hier de zogenaamde ovotestis en deze afwijkende gonade wordt gevonden in 54% van alle onderzochte gonaden van kinderen met OT-DSD.

Hoofdstuk 5 geeft, op grond van weefsel onderzoek (histologie), een beschrijving van drie verschillende vormen van de ovotestis. Deze vormen zijn niet eerder in de literatuur beschreven, ze hebben een belangrijke invloed op het behandelplan van kinderen met OT-DSD.

In **hoofdstuk 6** worden de histologische gegevens van verkregen biopsieen van gonaden vergeleken met de histologie van volledig verwijderde gonaden. Lang niet altijd wordt in de biopsie van de ovotestis testiculair en ovarieel weefsel gevonden. In een aantal biopten werd uitsluitend testiculair weefsel gezien, terwijl later bij onderzoek van de in zijn geheel verwijderde ovotestis zowel ovarieel als testiculair weefsel werd aangetoond. Op grond van deze bevindingen is een verbeterde biopsie techniek ontwikkeld. Dit houdt in dat er in de lengterichting over de gehele gonade een wigvormige biopsie wordt genomen. Op deze wijze is er een veel grotere kans op het aantreffen van, indien aanwezig, beide histologische componenten van de gonade (ovarieel en testiculair weefsel) en blijft er toch voldoende gonade weefsel achter voor de noodzakelijke hormonale en reproductieve functies.

Hoofdstuk 7 gaat over de verschillende methoden die er zijn om de inwendige genitalia te inspecteren en eventueel te biopteren. De laparoscopische techniek (kijkoperatie) wordt vergeleken met de zogenaamde open techniek (operatiewond). Geconcludeerd wordt dat de laparoscopische methode duidelijk voordelen heeft: het is minder belastend voor de patiënt, het geeft een goed zicht op de gonade en het nemen van een adequate biopsie is zeer goed mogelijk.

Deel 3 van het proefschrift bestaat uit 4 hoofdstukken over verschillende behandelaspecten van OT-DSD.

De functie van de gonade bij OT-DSD patiënten wordt besproken in **hoofdstuk 8.** Het is van groot belang om vast te stellen wat er met ovarieel en testiculair weefsel in dezelfde patiënt gedaan moet worden. De conclusie is dat ovarieel weefsel bij kinderen met OT-DSD op latere leeftijd hormonaal kan gaan functioneren. Om die reden dient het niet verwijderd te worden totdat het geslacht van het betreffende kind definitief is komen vast te staan. Daarentegen verliest testiculair weefsel zijn hormonale functie al in het eerste levensjaar en de testis kan later in een tumor ontaarden.

De follow-up van deze patiënten verloopt vaak uiterst moeizaam. Veel patiënten worden niet regelmatig voor controle op de polikliniek terug gezien. Deze slechte follow-up mogelijkheden, tezamen met de beperkte hormonale functie en kans op maligne ontaarding van de ovotestis, rechtvaardigen het verwijderen ervan, liefst in de eerste levensjaren. Hetzelfde kan gezegd worden van een testis indien het kind niet als jongen wordt opgevoed.

De chirurgische behandeling van kinderen met OT-DSD komt aan de orde in **hoofdstuk 9.** Alle vormen van chirurgische handelen moeten er op gericht zijn dat het kind kan opgroeien in het voor hem of haar passende geslacht, zonder risico te lopen op verandering van het geslacht op latere (postpuberale) leeftijd. Het wordt aanbevolen om de ouders te motiveren om regelmatig met hun kind voor controle te komen, omdat vaak op latere leeftijd nog specifieke reconstructieve chirurgische correcties nodig zijn.

Deze op latere leeftijd uit te voeren chirurgische reconstructies worden besproken in **hoofdstuk 10**. De voor- en nadelen van de verschillende chirurgische ingrepen alsook het beste tijdstip voor die ingrepen komen hier aan de orde.

De lichamelijke en psychosociale dilemma's waaraan deze patiënten gedurende hun groei en ontwikkeling worden blootgesteld en het belang hiervan voor het behandelplan, vormen belangrijke onderwerpen van **hoofdstuk 11**. Het is van groot belang om de ouders van kinderen die geboren worden met een onduidelijk geslacht zo volledig mogelijk te informeren over alle mogelijkheden en onmogelijkheden van behandeling en de complicaties die kunnen optreden. Alle bij de behandeling betrokken (para) medische specialisten moeten zich realiseren dat de informatie die aan de ouders gegeven gaat worden ook voor de ouders begrijpelijk moet zijn, slechts dan kunnen zij een weloverwogen informed consent geven. De beste zorg kan gegeven worden door een multidisciplinair team waarin alle behandelaren vertegenwoordigd zijn. Indien een dergelijk team niet ter beschikking is, moet de behandelend specialist terughoudend zijn met chirurgisch ingrijpen, tenminste tot het kind in de puberteit is en oud genoeg is om zelf beslissingen te nemen.

Besluitvorming omtrent de behandeling van kinderen met onduidelijk geslacht moet niet langer plaats vinden op een paternalistische wijze, dat wil zeggen uitsluitend door de behandelend specialist, maar moet gebaseerd zijn op input van het complete multidisciplinaire behandelteam waarbij rekening moet worden gehouden met de motivatie van de ouders. Op deze wijze kunnen kinderen met deze vaak gecompliceerde afwijkingen de beste zorg ontvangen.

Deel 4 van dit proefschrift betreft een algemene discussie waarin de belangrijkste aspecten van OT-DSD besproken worden in relatie tot de relevante bevindingen verkregen uit de voorafgaande hoofdstukken.

De onderzoekresultaten vermeld in dit proefschrift hebben voor kinderen met OT-DSD in Zuid Afrika geleid tot het:

- o vaststellen van een diagnostisch protocol;
- o laparoscopisch nemen van representatieve biopten van de gonaden;
- o beschrijven van een histologische classificatie van de ovotestis;
- o vaststellen van een behandelprotocol voor kinderen met OT-DSD.

Tenslotte wordt aangegeven op welke gebieden de kennis van OT-DSD nog onvolledig is en waar toekomstig onderzoek op gericht moet zijn.

List of Publications

About the author

Acknowledgements

LIST OF PUBLICATIONS

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ABOUT THE AUTHOR

Rinus Wiersma was born on the 26th March 1946 in Amsterdam, the Netherlands. At the age of 12 1/2-years his family emigrated to Southern Rhodesia (Zimbabwe), where he completed his grammar schooling. In 1966 he commenced his university education at the University of Natal (KwaZulu-Natal), Durban, South Africa, and completed his BSc. (Zoology) in 1970, after a change to the more sedate & studious environment of the university's Pietermaritzburg campus.

In 1971 he started his medical education at the University of Rhodesia (Zimbabwe) and graduated with a MB,ChB. in 1976. During his university vacations he worked in his home-town hospital laboratory, where he met a rather charming haematology technologist. He married Ann Helen Lowry on 23rd May 1975.

Following an internship and senior house officer's rotation, he worked for the final two years of the Zimbabwean liberation struggle in a rural hospital (Mutari, 1978-1980), attending to the civilian population and war-time victims. At the end of the war, and after a short surgical rotation in the Parirenyatwa General Hospital, Salisbury (Harare) he and his wife emigrated to South Africa. Here in 1981 he started his general surgical training at the University of Natal (KwaZulu-Natal), Durban, and completed with a Surgical Fellowship from the Royal College of Physicians and Surgeons of Glasgow in May 1985.

In 1986 he joined the Department of Paediatric Surgery, University of Natal as a surgeon / junior lecturer under the supervision of Prof.RE.Mickel, and advanced to the principal paediatric surgical position in 1992. After his initial two years of peadiatric surgical training, Mr.Wiersma worked for three months at the (old) Sophia Kinderziekenhuis, Rotterdam under the guidance of Prof.dr.JC.Molenaar, to gain invaluable experience in the management of paediatric surgical patients in the sophisticated hospital settings of a developed country.

Under the guidance of Prof.RE.Mickel, work was started in 1984 looking into the problems of children with ambiguous genitalia. This was an on-going study, which continued during his entire paediatric surgical career. In 2000 this resulted in a Master of Medical Science Degree from the University of Natal and in 2011 this thesis.

His research has addressed a variety of clinical problems encountered in his paediatric surgical practice. He has produced 28 published articles, 16 abstracts and 25 oral presentations for national and international paediatric surgical forums. The subjects of his research have highlighted his wide interest in paediatric surgical problems of the Third World, the unique problems of congenital abnormalities, disorder of sex development, paediatric urology, paediatric trauma, HIV and many of the infective conditions seen in our South African children.

Between 1994 and 2009 Mr.Wiersma spend several periods of sabbatical leave at the Sophia Kinderziekenhuis under the guidance of Prof.dr.FWJ.Hazebroek. This allowed discussion of new ideas of the paediatric surgical management with colleagues and resulted in the introduction of many of these ideas into his own practice.

Mr.Wiersma has served on several medical bodies. In 2002 he was elected onto the committee of the South African Association of Paediatric Surgeons (SAAPS), and in August 2004 he was elected onto the Executive Committee and served as Secretary/Treasurer for a period of two years. He was appointed for a five-year period (2003-2007) as one of five part-time Hospital Domain Managers to clinically assist with the running of the new Inkosi Albert Luthuli Central Hospital in Durban. He has been an external examiner for the South African College of Surgeons Fellowship examinations (1998-2006), as well as for the Diploma in Primary Emergency Care of the College of Family Practitioners examinations (2003-6). In 2010 he was appointed to assist the International Olympic Committee on their deliberations on Disorders of Sex Development in Miami, USA, and still serves on this body an *ad-hoc* basis.

Mr.Wiersma is still married, they have two sons Paul & Nick both of whom work in the IT industry.

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To my wife **Ann Wiersma (nee Lowry)**, I count myself true blessed to have you by my side on our journey through life. We complement each other and share everything, but your ever patient tolerance of, and guidance in my academic 'other life' is amazing. You have always been a great sounding board for my many projects and articles. Without your solid support behind me, some battles would not have been won and this thesis would not have seen the light of day.

To the parents and children who have suffered ovotesticular disorder of sex development over the years. Hopefully my attempts to find some of the answers to their many problems has made the lives of some past patients more tolerable and the lives of future patients more acceptable.

My deepest appreciation goes to **Ms. E. Conner** for her untiring administrative work. Eleanor, you are the hub the paediatric surgical department, little works without your input. You have been a real friend to me at work and your support and efforts with my papers over the many years has been fantastic. Thank you!

This is my second attempt of this thesis. The first was a compilation of past papers and lectures on the subject of ovotesticular DSD, promoted by **Prof. Sandy Thomson** and **Prof. David Muckart** here at the University of KwaZulu-Natal. The thesis had been accepted in principal through senate and written. Unfortunately due to an administrative glitch no registration number had been awarded, which caused the thesis in its final draft to be rejected. However the work that went into it by both Sandy and David was immense. I would like thank you both for your insight and support of my work. Your hard work, unfortunately, did not come to any conclusion, but should not go without recognition. To you both I am truly grateful.

Prof.GP.Hadley, Professor and chief of the Department of Paediatric Surgery, University of KwaZulu-Natal, thank you for your assistance over the years. You and I have been through some amazing years at work. Lots of work with few staff members, but experiences that have been truly incredible and thought provoking. The past 25 years have been an incredible ride with many memorable experiences that have made us into better doctors and people.

There are many people who have provided me with assistance along the way, but have not been mentioned here by name. I salute and thank all of you who have provided me with inspiration, information and useful suggestions. To be a good doctor one has to be a good listener first.

Yours
Rinus Wiersma