

## Obtaining a Diagnosis

Investigation, diagnosis and corrective measures for the eunuch population is rarely done in India. These measures are more prevalent in Western countries, much less stigma attached to this 'deviant sex' and consequently, remedial procedures are the norm. There, the affected person stands a much greater chance of being able to undertake proper surgical, hormonal and other rehabilitation therapy and lead a life according to the desired gender.

Diagnosis of AIS is usually made at one of these life stages:

- As child – following the detection of hernia or 'non-standard' genitalia.
- As an adolescent – with the failure to start menstruating.
- As an adult – trying to piece together her medical history and often uncovering lies/half-truths over many years.

The first test to be carried out in a suspected case will probably be one which examines whether XY sex chromosomes are present. These are often detected either via a chromatin test using a buccal (mouth) smear (a rough indication that looks for a particular colour change in cells under a microscope) or via a blood test (a karyotype test that examines the shape of the actual chromosomes). There is also a new smear test called *Fluoro In Situ Hybridisation (FISH)* in which the X chromosome shows up as green and the Y as red.

Patients and their parents are often traumatized enough by the existence of XY chromosomes that they settle at a diagnosis of 'AIS' and do not pursue further tests to differentiate similar conditions. Nevertheless, it is quite useful to investigate further, particularly in the case of suspected *Partial AIS (PAIS)*, which has a presentation similar to several other conditions. In such cases, the following tests may be done.

### Investigations in PAIS

There are other conditions, apart from AIS, that will produce the same type of genital 'deformity' in a genetic male but the body's sensitivity to androgens can be tested in infancy by applying testosterone ointment to the pubic region. If no response or only a weak response to the hormone is observed, then the baby has PAIS and a female assignment should be more strongly considered. At puberty, the patient will probably develop breasts and be unable to virilize completely. Also, the phallic enlargement that normally occurs at puberty may not occur, so that a penis that is marginal in size will remain so.

X-ray studies involving a radio-opaque dye injected into the urogenital sinus can be used to determine the extent of development of the vagina. The vagina and labia minora may be fairly well-developed but not evident on external examination due to labial fusion.

### **Blood Tests**

- Karyotype (done on everyone)
- 17-hydroxy-progesterone (if Congenital Adrenal Hyperplasia, an XX intersex condition, suspected)
- Testosterone
- Dihydro-testosterone (DHT)
- Androstenedione
- HCG (human chorionic gonadotropin) stimulation test (3-day or 3-week test). By administering human chorionic gonadotropins (similar to pituitary hormones) and checking for resulting androgen production, it is possible to check whether a patient suspected of having AIS might instead have a deficiency in androgen production rather than of deficiency in response to androgens.
- Gonadotropins (i.e. FSH – Follicle Stimulating Hormone and LH - Luteinizing Hormone)

### **Urine Tests**

Urinary steroids may be measured (not always very useful) but may help with 5-alpha-reductase. 5-alpha reductase, also known as 3-oxo-5-alpha-steroid 4-dehydrogenase is

an enzyme involved in steroid metabolism. It participates in 3 metabolic pathways: bile acid biosynthesis, androgen and estrogen metabolism and prostate cancer.

## **Imaging**

- Ultrasound of pelvis for uterus, ovaries. These tests check for the presence or absence of internal Mullerian (female) structures and differentiate AIS from conditions such as Swyer's Syndrome, gonadal dysgenesis, MRKH (Mayer Rokitansky Kuster Hauser Syndrome).
- Ultrasound of groins for testes.
- Patient may also have an MRI scan if above is not found useful.
- Sinograms (not really done now but used to be done to look for a vagina).

## **Examination under Anesthetic**

This may be done if imaging cannot give the whole answer or to plan for surgery. It may occasionally include a laparoscopy (examination of abdominal contents via telescopic device through small incision).

## **Genital Skin Biopsy**

Used to be done just for research but now may be done to look for DNA (although this can also be done using blood).

## **Gonadal Biopsy**

An examination of the testes being removed; in cases of AIS these tissues are supposed to look normal and be functioning (although they do not produce mature sperm cells). In other cases the gonads may present as streaks, as under-developed or even as ovo-testes. This test is rarely done now unless looking to confirm true hermaphrodite i.e. the presence of an ovo-testis.

## **Gonadal Histology**

If the gonads are removed they may be sent for histological analysis (to look at the structure at cellular level). This may sometimes help in the diagnosis e.g. in Leydig cell hypoplasia.

### **DNA Studies**

Usually done on blood. In some conditions, e.g. Complete AIS, the faulty gene can be identified in about two thirds of people with this condition. This means that if identified, other members of the family could be screened to see if they carry it too (precautionary). Only a few centres can offer this, all in the western world. The AIS gene is done in Cambridge UK, 17-keto-steroid in Dallas USA and France. Most centres are however happy to send off blood for this test if there is a good chance the result will be useful. They are very expensive and time-consuming 'needle in a haystack' tests. The clinical suspicion has to be very high to make it worthwhile.

### **Anti-Müllerian Hormone (AMH)**

Anti-Müllerian hormone also known as AMH is a protein that, in humans, is encoded by the AMH gene. It inhibits the development of the Müllerian ducts in the male embryo. It has also been called Müllerian inhibiting factor (MIF), Müllerian inhibiting hormone (MIH), and Müllerian inhibiting substance (MIS). AMH prevents the development of the mullerian ducts into the uterus and other mullerian structures. Each testis suppresses Müllerian development only on its own side. In humans, this action takes place during the first 8 weeks of gestation. If no hormone is produced from the gonads, the Mullerian ducts automatically develop, while the Wolffian ducts, which are responsible for male reproductive ducts, automatically die

This test not available in India but widely used in the United States. It is a good marker for the presence of a testis.

### **Familial Analysis**

While about a third to a half of cases of AIS occur due to spontaneous mutations, the other cases follow a specific line of inheritance, namely through maternal lines. In cases

where a relative with a similar condition is known on a *non-maternal* line or when there is consanguinity (close blood relationship) between parents, one may suspect that the girl does not have AIS but another condition with an *autosomal* inheritance (i.e. inheritance that does not involve either of the sex chromosomes but one of the 46 other chromosomes).

### **Prenatal Diagnosis**

AIS has been diagnosed as early as 9-12 weeks of intrauterine life by chronic villus sampling (sampling of tissue from the foetal side of the placenta). By the 16th week it can be detected by ultrasound and amniocentesis (sampling and analysis of amniotic fluid). However, pre-natal diagnosis is seldom prescribed unless there is a family history of the condition.

### **Tissue-Level Defect**

The exact nature of the defect at the tissue level in AIS has been the subject of considerable research. A recent paper from these researchers studies the molecular basis of the phenotypic (body appearance) variation in AIS. They put forward evidence that most, if not all, cases of complete AIS can be explained by androgen receptor defects but that the majority of Partial or Incomplete AIS subjects exhibit no defect in androgen-receptor binding, suggesting that other genetic defects are involved. It is also possible to have the same genetic defect and different genital appearances. CAIS and PAIS may thus be caused by different defects at the genetic/cellular level and are thought not to occur in the same family.

### **Carrier Detection**

It is possible to detect, through biochemical means, XX females in a family who may be carriers of the faulty gene. Recent work by the Cambridge research group in the UK on the partial form of AIS concludes that although PAIS carrier status can be determined in

fertile females in a family, the severity of genital abnormalities in affected offspring cannot be reliably predicted.

At one time, when the androgen receptor gene was being actively researched, it was possible to get research labs to carry out carrier testing on behalf of families, alongside their research. However interest in and hence funding for this research has faded since most of the androgen receptor gene defects have now been discovered. So although a lot of research work has been done in the UK on the genetics of AIS, no routine carrier testing service has resulted from this, as far as we know, although there are various commercial testing laboratories in the US and continental Europe, but none in India. The number of families requiring this service is, of course, small since AIS is a rare phenomenon but the demand has increased in recent years and will probably continue to grow, as intersex becomes less of a secret issue within families and society due to the integration offered by globalization and Indian society just starting to become more open and accepting like Western cultures.

In the absence of biochemical testing, the following clues to ascertain presence of carrier status in a family can be used:

- maternal relatives affected by AIS
- delayed puberty in an XX female
- reduced pubic/axillary hair in an XX female
- asymmetric pubic/axillary hair in an XX female
- reduced bone density in an XX female.

### **Diagnostic criteria for Gender Identity Disorder**

A. A strong and persistent cross-gender identification (not merely a desire for any perceived cultural advantages of being the other sex). In children, the disturbance is manifested by four (or more) of the following reasons:

1. Repeatedly stated desire to be or insistence that he or she belongs to the other sex.
2. In boys, preference for cross-dressing or simulating female attire; in girls, insistence on wearing only stereotypical masculine clothing.

3. Strong and persistent preferences for cross-sex roles in make-believe play or persistent fantasies of being the other sex.
  4. Intense desire to participate in the stereotypical games and pastimes of the other sex.
  5. Strong preference for playmates of the other sex. In adolescents and adults, the disturbance is manifested by symptoms such as a stated desire to be the other sex, frequent passing as the other sex, desire to live or be treated as the other sex or the conviction that he or she has the typical feelings and reactions of the other sex.
- B. Persistent discomfort with his or her sex or sense of inappropriateness in the gender role of that sex. In children, the disturbance is manifested by any of the following: in boys, assertion that his penis or testes are disgusting or will disappear or assertion that it would be better not to have a penis or aversion toward rough-and-tumble play and rejection of male stereotypical toys, games and activities; in girls, rejection of urinating in a sitting position, assertion that she has or will grow a penis or assertion that she does not want to grow breasts or menstruate or marked aversion toward normative feminine clothing. In adolescents and adults, the disturbance is manifested by symptoms such as preoccupation with getting rid of primary and secondary sex characteristics (e.g., request for hormones, surgery or other procedures to physically alter sexual characteristics to simulate the other sex) or belief that he or she was born the wrong sex.
- C. The disturbance is not concurrent with a physical intersex condition.
- D. The disturbance causes clinically significant distress or impairment in social, occupational or other important areas of functioning.