Chapter
2

Primary Amenorrhoea

LEARNING OBJECTIVES

- To understand the basics of sexual differentiation.
- To know the causes of primary amenorrhea and its evaluation.
- To describe the management of the individual condition.
- To know the recent developments and fertility preservation options in the management of primary amenorrhoea.

BASICS OF SEXUAL DIFFERENTIATION

Before discussing the causes and evaluation of primary amenorrhoea, it is important to understand the basics of sexual differentiation and pubertal changes.

Sexual Differentiation

Sexual differentiation includes chromosomal sex, gonadal sex, differentiation of internal genital organs and external genital organs and sex of rearing.

Chromosomal Sex

At fertilization, the haploid gametes unite and the conceptus contains 46 chromosomes; 22 autosomes are derived from each of the ovum and sperm, the ovum donating one X chromosome and the sperm either one X or a Y. The 46 XX embryo differentiates into a female, whereas the 46 XY embryo becomes a male.

It is the presence or absence of the Y chromosome which determines whether the undifferentiated gonad becomes a testes or an ovary.

Gonadal Sex

Although the chromosomal sex is determined at the time of fertilization, the gonadal sex is complete only around the 10th week of development and this results from the differentiation of the indifferent gonad to become either a testes or an ovary. Around the 5th week of development, an area of coelomic epithelium develops on the medial aspect of the urogenital ridge, which later becomes the gonadal ridge. Epithelial cords (primary sex cords) now grow into the mesenchyme and the gonad now possesses an outer cortex and an inner medulla (Fig. 2.1).

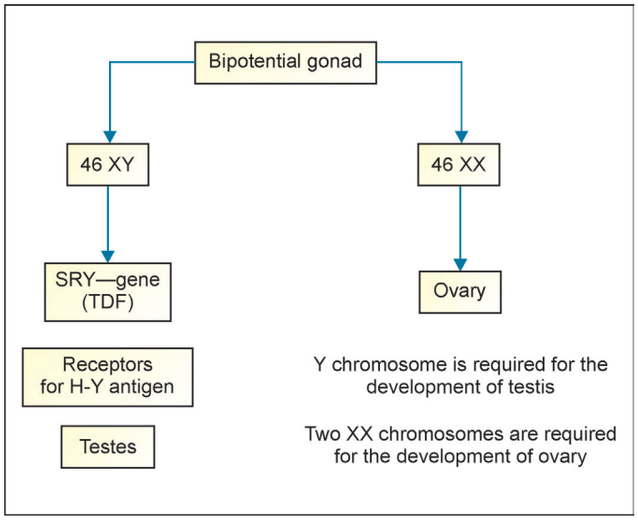

Fig. 2.1: Gonadal development

- In XY individuals, the medulla becomes the testes and the cortex regresses. Whereas, in XX individuals, the cortex differentiates to become the ovary and the medulla regresses.
- The primordial germ cells develop by the 4th week in the endodermal cells of the yolk sac and during the 5th week, they migrate along the dorsal mesentery of the hindgut to the gonadal ridges, eventually incorporating into the mesenchyme and primary sex cords by the end of 6th week.

Development of the Testes

The primary sex cords become concentrated on the medulla of the gonad and proliferate and their ends anastomose to form the rete testes. The developing sex cords become the seminiferous tubules, and the

mesenchyme grows between the tubules to separate them (Leydig cells). The seminiferous tubules are composed of two layers of cells; supporting cells (Sertoli cells) derived from the germinal epithelium and the spermatogonia derived from the primordial germ cells.

Development of the Ovary

The development of the ovary is much slower than that of the testes and the ovary is not evident until the 10th week of gestation. Now the primary sex cords regress and finally disappear. Around the 12th week, secondary sex cords arise from the germinal epithelium and the primordial germ cells become incorporated into these cortical cords. At 16 weeks, these cortical cords break to form an isolated group of cells called primordial follicles; each cell contains an oogonium derived from the primordial germ cells, surrounded by follicular cells arising from the cortical cords. These oogonia undergo rapid mitosis to increase the number to thousands of germ cells called primary oocytes. Each oocyte is surrounded by a layer of follicular cells, the whole structure is now called the primary follicle. The surrounding mesenchyme becomes the stroma.

Genetic Control of Gonadal Development

The genes *LIM1*, *WT1* and *FTZ-F1* have been shown to be involved in the formation of the gonads prior to their differentiation as testes or ovaries. Subsequently, sex-specific gonadal differentiation is mediated by the *SRY* and *SOX9* genes in the testis, and the *DAX*-1 gene in the ovary.[1]

DEVELOPMENT OF THE INTERNAL AND EXTERNAL GENITAL ORGANS

Both sexes develop two pairs of genital ducts; the Wolffian ducts (mesonephric ducts) and müllerian ducts (paramesonephric ducts). The Wolffian ducts arise in the mesonephros that run caudally to enter the urogenital sinus near the müllerian tubercle. The müllerian ducts develop laterally to the Wolffian ducts run inferiorly and parallel to the Wolffian ducts and at the caudal end it cross the wolffian ducts anteriorly and fuses in the midline and enter the urogenital sinus forming the müllerian tubercle.

Development of Male Genital Organs

Development of the male internal structures require regression of the müllerian ducts by the müllerian inhibiting factor secreted by the testes, primarily by the Sertoli cells. This is mediated through the release of hyaluronidase by the müllerian duct cells, thus leading to local destruction. The Wolffian ducts develop under the stimulation of testosterone resulting in epididymis, vas deferens and seminal vesicles. The urogenital sinus undergoes masculinization; the penis forms from the müllerian tubercle, elongation of the urogenital folds results in urethra and scrotum forms from the fusion of the labioscrotal swellings (Fig. 2.2).

Development of Female Internal and External Genital Organs

The open ends of the müllerian ducts become the fallopian tubes. The fused portion gives rise to the epithelium and glands of the uterus. The myometrium is derived from the surrounding mesenchyme. At the point of fusion, a uterine septum is present which later regresses to form a single cavity. The fusion of the müllerian ducts brings two peritoneal folds towards the midline forming the broad ligaments (Fig. 2.3).

- Vagina develops from two sources; uterovaginal canal and sinovaginal bulbs in the urogenital sinus. As the uterovaginal canal reaches the pelvic portion of the urogenital sinus, there is development of two outgrowths called sinovaginal bulbs. They fuse to form a solid vaginal plate which grows rapidly in the cranial direction. This eventually cavitates beginning caudally and by 20 weeks, the vagina is fully formed. The hymen represents the remnants of the müllerian tubercle. Wolffian structures regress and the remnants remain as hydatid cyst of Morgagni and Gartner's cyst.
- Formation of external genitalia: Müllerian tubercle forms the clitoris, urogenital folds form the labia minora and the labioscrotal folds form the labia majora.

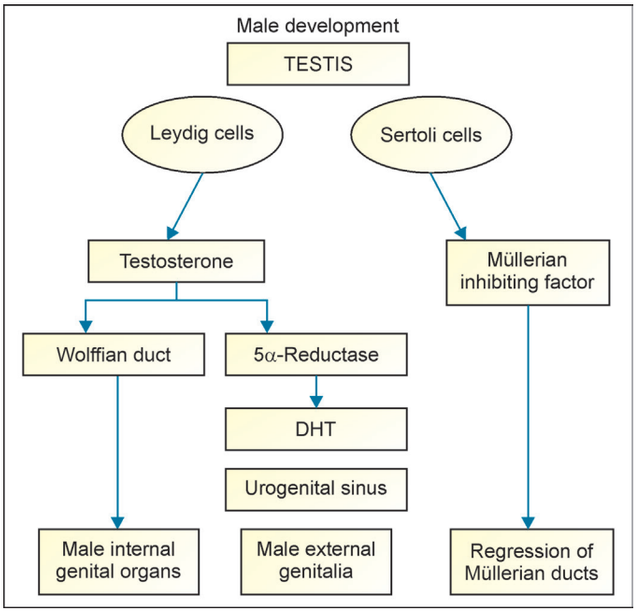

Fig. 2.2: Development of male internal and external genital organs

Normal sexual differentiation depends on the following features

- Two X chromosomes are necessary for normal ovarian development.
- Absence of gonad results in female phenotype.
- In the presence of the ovary or when there is no gonadal development, the individual develops into a female which is a neutral development. Due to the absence of müllerian inhibiting factor, the female internal genital organs develop and due to the absence of androgen exposure, the external genitalia develop into a female (Fig. 2.3).
- H-Y antigen and testes determining factor controls the evolution of gonad to become the testes in the presence of an XY chromosome.
- Sertoli cells produce müllerian inhibiting factor and this leads to the disappearance of müllerian ducts.
- Fetal testosterone induces Wolffian duct development.
- Masculinization of the cloaca can occur only if testosterone is bound to a receptor and converted to dihydrotestosterone.
- Absence of 5α-reductase will fail to allow masculinization of the cloaca, but Wolffian development will be normal.
- Exogenous androgen exposure during cloacal development in the female will masculinize the cloaca, but has no effect on müllerian structures.

Basic factors involved in the initiation and continuation of normal menstruation

- Normal female chromosomal pattern 46 XX
- Normal development of the ovary
- Co-ordinated HPO axis functioning
- Anatomical patency of the genital tract
- Active support of the adrenal and thyroid glands
- Responsive endometrium

PUBERTY

Normal pubertal development is controlled by the hypothalamus and is dependent upon the GnRH release from the hypothalamus, gonadotrophin and growth hormone secretion from the pituitary and normal functioning of the hypothalamopituitary gonadal axis. Most changes during puberty are gradual, usually occurring in regular sequence between the ages of 10 and 16 years and the onset of menstruation is one manifestation of puberty.

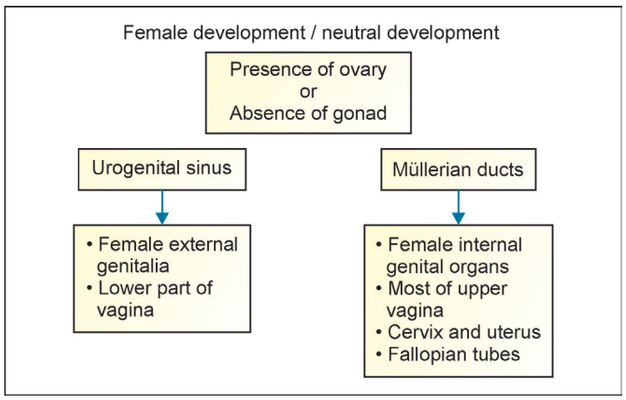

Fig. 2.3: Development of female internal and external genital organs

Phases of Pubertal Growth

There are 4 different phases of pubertal growth (Table 2.1).

Phase I

The first physical sign of puberty is breast budding (thelarche) which corresponds to increase in ovarian hormone secretion in response to GnRH stimulation (gonadarche). Breast changes begin around 9 years of age, go through 5 stages (Tanner staging) in sequence and the full development takes 5 years (*refer* to Fig. 1.1, Chapter 1)

Phase II

Thelarche is followed by adrenarche (pubarche) which is the first indicator of adrenal androgen secretion; DHEA, DHEA-S and androstenedione. These hormonal changes result in pubic hair development followed by growth of axillary hair. Acne, body odour and oily skin are the other sequelae of adrenarche.

Phase III

In this phase there is maximum growth spurt or peak growth velocity which is seen 2 years earlier in girls than in boys. This growth spurt occurs about one year prior to menarche.

Phase IV

In phase IV, menarche, the first menstruation occurs.

Tanner Staging

Tanner scale was first identified in 1969 by James Tanner, a British paediatrician, after a two-decade-long study following the physical changes in girls undergoing puberty.[2] Tanner stages are used to describe the sequence of development of breast and pubic hair, beginning with stage 1 (pre-pubertal) and concluding with stage 5 (adult). In addition, axillary hair is graded from stage 1 (pre-pubertal) to stage 3 (full adult development).

Table 2.1: Phases of pubertal growth

Thelarche (gonadarche)	10–11 years
Adrenarche (pubarche)	11–12 years
Peak height or growth velocity	12–13 years
Menarche	13–14 years
Mature sexual hair and breast	14–15 years

Pubic hair scale and female breast development scale have been discussed in detail in Chapter 1 on precocious puberty. At the time of sexual maturation, there is also increased somatic growth due to increase in the growth hormone levels which directly or indirectly through the liver, increases the insulin-like growth factor 1 (IGF-1) which induces somatic growth (Fig. 2.4). The pattern of gonadotrophin secretion and the steroid biosynthesis are shown in Table 2.2.

PRIMARY AMENORRHOEA

Introduction

Primary amenorrhoea is defined as the absence of menstruation in a girl by 16 years of age. However, different criteria have also been used to define primary amenorrhoea. As the pubertal changes precede menarche, some authors define primary amenorrhoea as an absence of *secondary sexual characteristics* by age 14 with no *menarche* or normal secondary sexual characteristics but not attained menarche by 16 years of age.[3]

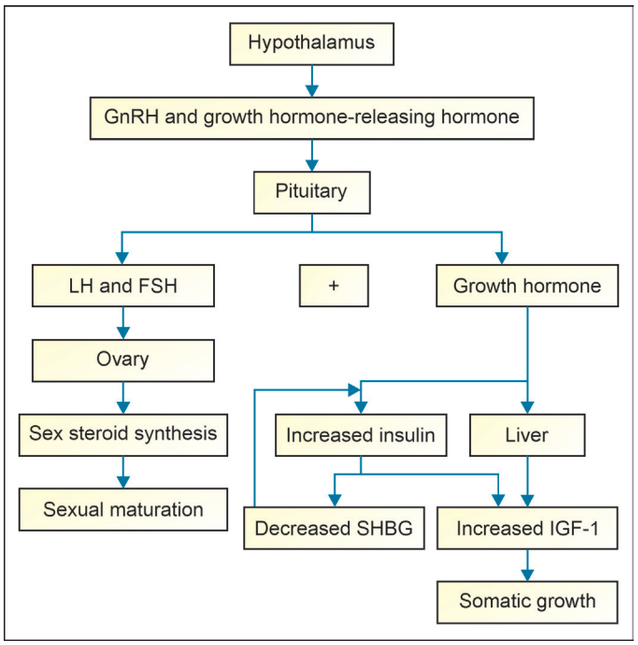

Fig. 2.4: Endocrine changes associated with pubertal and somatic development

The mean age of menarche is 13.5 years, but the age of menarche can vary between and within the countries. Age of menarche can vary with race, genetic factors, social class, family size, birth order, nutritional status, general health, physical activity, environment and climate. Generally, girls from rural areas in low-resourced countries are older at menarche than girls from urban areas.[4,5] Obese individuals attain menarche earlier than non-obese girls. This is explained by the *critical body weight hypothesis*. On reaching the critical body weight, especially the body fat, there are metabolic changes are leading to menarche. In obese individuals, there is an increase in insulin levels which in turn increase IGF-1 and decrease in SHBG, thereby increasing the bioavailability of steroids leading to early menarche. Leptin, a hormone found in adipose tissue, has direct action on hypothalamus leading to increased levels of insulin, and increased pulsatility of GnRH. Increased leptin levels are associated with increased body fat.

Individuals presenting with primary amenorrhoea suffer from profound physical and psychological morbidity. They need detailed evaluation, management and psychological support. The goals of management are to facilitate normal sexual life, restore menstrual function, and improve the chances of childbearing in suitable candidates. It is important to provide them with a stable sex of rearing. It is also important to take preventive measures for osteoporosis and other medical problems. Gonadectomy plays a major role in preventing malignancy in XY individuals.

When to Investigate for Primary Amenorrhoea?

Though one should initiate investigations for primary amenorrhoea if the individual has not attained menarche

Table 2.2: Hormonal changes associated with pubertal development

At 20 weeks of intrauterine life	Fetal FSH/LH levels rise to reach adult concentration
3 months of neonatal life	Due to the loss of maternal and placental steroids, there is reflex stimulation of the HPO axis leading to a second peak of gonadotrophin levels
4–10 years of childhood	The HPO axis is suppressed due to intrinsic CNS inhibition, and there is increased sensitivity to negative feedback by very low oestradiol levels in pre-pubertal children Adrenal androgens DHEA and its sulphate increase as early as 6–7 years
11 years	There is a loss of sensitivity to negative feedback leading to nocturnal GnRH release
12 years	The nocturnal release of GnRH release is replaced by adult secretory pattern of GnRH pulses every 90–120 minutes, 24 hours a day. Circulating FSH levels increase approximately 1 year prior to those of LH. There is increase in growth hormone and secondary rise of DHEA levels
13 years	Adolescent FSH/LH secretion and secretion of sex steroids
14 years	Ovulatory cycles

by her 16th birthday, in certain situations, investigations should be initiated early. As there is also a trend towards earlier age at menarche, early evaluation is recommended by many authors.[6] Recent data suggest that pubertal development, and menarche, begin earlier in American girls. Consequently, some clinicians would consider initiating an evaluation of a girl for primary amenorrhoea by age 14, particularly if 5 or more years had passed since the first evidence of pubertal development.[7]

Indications for Early Evaluation

1. Failure of development of breast and/or secondary sexual characteristics by 13–14 years of age.
2. When there is evidence of virilisation with hirsutism and clitoromegaly.
3. When the individual is short-statured. The height increases at a constant rate of 6 cm/year from the age of 2 until the onset of puberty. During puberty there is accelerated growth with a peak height velocity of 11 cm/year around 10–14 years.
4. When the individual presents with cyclical abdominal pain with retention of urine and/or difficulty in defaecation (these may be the presenting symptoms in cryptomenorrhoea or Mayer-Rokitansky-Küster-Hauser syndrome (RKHS), where müllerian knobs may contain active endometrial tissue resulting in haematometra/or endometriosis).
5. Headache and visual disturbances may be the first presenting symptom when pathology in the brain is the cause of primary amenorrhoea.

Causes of Primary Amenorrhoea

Causes of primary amenorrhoea can be broadly classified based on the site of pathology is summarized in Table 2.3.

EVALUATING A CASE OF PRIMARY AMENORRHOEA

History

A detailed history and a thorough examination will give a clue to the possible cause of primary amenorrhoea in most cases. This initial step will also help the clinicians in deciding the relevant investigations that would be required in the index case, rather than ordering a battery of investigations.

Demographic details

- Age of the patient
- Education, occupation
- Socioeconomic status
- Marital history: Married/Single

In utero history

- Details of exposure to teratogens when the patient was in her mother's womb may give a clue to the possible aetiology of primary amenorrhea. *In utero* history should be elicited from her mother, or if possible from the antenatal case records of the mother.
- Age of the mother when the patient was born.
- Any drugs taken by the mother during pregnancy, especially hormones.
- Whether the mother suffered from a threatened miscarriage.
- Whether there was any X-ray exposure/general anaesthesia during early pregnancy.
- Any other acute illness during early pregnancy.
- Whether any possible antepartum and intrapartum insults and injuries leading to hypoxia.

Childhood history

The performance of a child at school, its growth pattern and various illness acquired by the child may give a clue to the possible involvement of the central nervous system or involvement of the gonads.

- Milestones
- Intelligence/mental retardation: Low IQ/mental retardation are seen in CNS disorders such as Laurence-Moon-Biedel syndrome and Prader-Willi syndrome. Low IQ may also be seen in chromosomal problems.
- School performance, personality, growth pattern
- Childhood illness:
  - A. History of Koch, meningitis, encephalitis, seizures, anorexia, headache and visual disturbances may indicate hypothalamic/pituitary causes of primary amenorrhoea.
  - B. History of viral infection such as mumps/rubella which can lead to viral oophoritis causing premature ovarian failure/ovarian agenesis.
  - C. Similarly, childhood tuberculosis and rheumatic fever can also affect the genital organs leading to ovarian failure.
  - D. Childhood malignancy such as leukaemia and use of chemotherapy and radiotherapy which can lead to loss of ovarian function.
  - E. History of head injury.

Pubertal changes

- Age of thelarche, adrenarche, growth spurt normal or delayed/virilising puberty.
- Whether history of periods induced with hormones.

Family history

Family history is very important, as a number of conditions causing primary amenorrhoea can run in families.

- History of genetic defects in the family (Table 2.4)
- Mother's age of menarche and menopause
  - Whether mother's puberty was normal/delayed menarche/premature menopause
- Sibling's history

Table 2.3: Causes of primary amenorrhoea

Chromosomal aberrations	• Turner's syndrome • Turner mosaic • Mixed gonadal dysgenesis with a chromosomal pattern of XO/XY
Gonadal problems	• Pure gonadal agenesis (also called pure gonadal dysgenesis). The chromosomal pattern is XX or XY • Resistant ovary syndrome (Savage syndrome) • Premature ovarian failure due to chemotherapy, radiotherapy, childhood malignancy, tuberculosis, mumps oophoritis, autoimmune disease, galactosaemia • Enzymatic deficiency such as aromatase deficiency and 17α-hydroxylase deficiency • True hermaphrodite • Virilizing male intersex due to lack of müllerian inhibitor and partial cloacal resistance to normal intrauterine androgen production • Masculinizing tumors of the ovary • Polycystic ovarian disease
End-organ resistance	• Androgen insensitivity syndrome (testicular feminization syndrome) • Cryptorchid male intersex
Pituitary disorders	• Pituitary tumours: Chromophobe adenomas, chromophil adenomas leading to acromegaly and Cushing's disease • Isolated gonadotrophin deficiency • Pan hypopituitarism due to empty sella syndrome • Mutations of FSH and LH receptors
Hypothalamic causes	• Trauma, hydrocephalus, encephalitis, meningitis, tuberculosis, sarcoidosis, tumours • Isolated GnRH deficiency (Greak coil syndrome) • Psychogenic—emotional shock and anorexia nervosa • Craniopharyngioma • Drugs—phenothiazines • Laurence-Moon-Biedel syndrome • Olfactogenital syndrome
Endocrine causes	• Adrenal causes • Congenital adrenal hyperplasia • Cushing's disease • Thyroid dysfunction • Hypothyroidism
Outflow obstruction	• Imperforate hymen • Transverse vaginal septum • Müllerian agenesis (Mayer-Rokitansky-Küster-Hauser syndrome) • Isolated vaginal agenesis
Constitutional delay	
Non-responsive endometrium	• Tuberculous endometritis leading to intrauterine adhesions
General causes	• Rheumatic fever, tuberculosis • Severe malnutrition, severe malabsorption • Diabetes, nephritis, cirrhosis, • Obesity • Leukemia • HIV

Table 2.4: Genetic defects associated with primary amenorrhoea

	Condition	***Genetic defect and mode of transmission***
1	Testicular feminization	X-linked recessive gene. Maternal aunts will be affected
2	Pure gonadal dysgenesis (gonadal agenesis). There are different modes of inheritance	Autosomal recessive (XX) gene X-linked recessive gene (XY) Autosomal dominant genes expressed in females
3	Congenital adrenal hyperplasia	Autosomal recessive gene
4	Müllerian agenesis	Autosomal dominant gene transmitted by the male relatives
5	Laurence-Moon-Biedel syndrome	Autosomal recessive transmission

  - History of primary amenorrhoea among siblings
  - What is the order of index patient among siblings?
  - Siblings whether fertile or subfertile and whether investigated for infertility?
- History in maternal aunts
  - In testicular feminization maternal aunts may be affected
- History of congenital anomaly in the family
- History of inguinal hernia in the family
- History of consanguinity
- Conditions such as pure gonadal dysgenesis and congenital adrenal hyperplasia are transmitted by an autosomal recessive gene, therefore, inheritance of the disease can result from consanguineous marriages. Several siblings can be affected in the same family.
- Family history of hypothyroidism/diabetes/autoimmune disorder in the family. Pedigree analysis has shown that Turner's syndrome can be a heredofamilial disorder. The patient with Turner's syndrome and/or her parents may suffer from diabetes/hypothyroidism or other autoimmune disorders.
- Family history of tuberculosis

Medical history

A detailed medical history should be taken for:

- Thyroid disorders—hypo- and hyperthyroidism
- Cirrhosis
- Hypothalamopituitary disorders—indicated by headache, giddiness, anosmia, visual disturbances, galactorrhoea, drugs causing hyperprolactinemia.
- Adrenals—obesity, hirsutism, pigmentation, virilisation, clitoromegaly, deepening of voice.
- Anaemia/malnutrition, chronic renal disease, rheumatic disease, psychiatric illness, HIV.

Past surgical history

History of abdominal surgery for ovarian cyst removal/management of tubo-ovarian abscess.

Personal history

- Excessive weight loss/weight gain
- Sleep/bowel/micturition disturbances
- Change in appetite and dietary habits
- Use of drugs/stress
- Occupation/whether an athlete
- History of excessive hair growth
- Presence of hot flushes

Sexual activity

- Whether involved in consensual sexual intercourse or is she a victim of sexual violence?
- Whether there is difficulty in sexual intercourse?

Previous treatment

- Hormones, anti-Koch, antipsychotic drugs
- Whether the individual has undergone examination under anaesthesia and endoscopy.
- Treatment for primary amenorrhoea and response to treatment.

EXAMINATION

Anthropometric measurements. The height and weight are measured and the BMI is calculated.

Height

Short stature is seen in:

a. Turner's syndrome due to loss of an X chromosome—as the genes responsible for height are lost
b. Turner's mosaic—due to loss of short arm of X chromosome
c. Pituitary dwarfism, cretinism
d. Hypothalamic damage caused by congenital hydrocephalus, trauma, empty sella syndrome and craniopharyngioma.

In the absence of oestrogen, patients are tall and epiphyseal closure does not occur.

Tall stature is seen in:

a. Pure gonadal dysgenesis as there is no oestrogen.
b. Testicular feminisation—the testosterone levels are normal or high with decreased oestrogen levels. These women have long arms reaching below the knee.
c. Hypogonadotrophic hypogonadism as there is no oestrogen secondary to absence or low gonadotrophins.
d. Mixed gonadal dysgenesis as there is no oestrogen.

Tall and gigantic stature is seen in acromegaly

In Turner and Turner's mosaic, though the oestrogen levels are low, the genes responsible for the height are lost. Therefore, they are short.

Measurement of Span

Normally the height is = to span or height is > span.

Span is taken from the tip of left middle finger to the tip of the right middle finger in a wide stretched arm (Fig. 2.5).

In Turner's syndrome, the span is > the height by at least 1–1.5 inches. Here the closure of epiphysis of the long bones of the arms occurs late due to lack of oestrogen.

Crown-rump and Rump Heel Measurement (Upper to Lower Limb Ratio (U/L Ratio)

The distance from the top of the pubic ramus to the top of the head is divided by the distance from the pubic ramus to the foot. The mean U/L ratio is 0.85 to 0.92. In hypogonadal patients, including Turner the U/L ratio is decreased.

Weight

Obesity is seen in pre-pubertal PCOS, hypothyroidism, Cushing's disease, pituitary and hypothalamic obesity

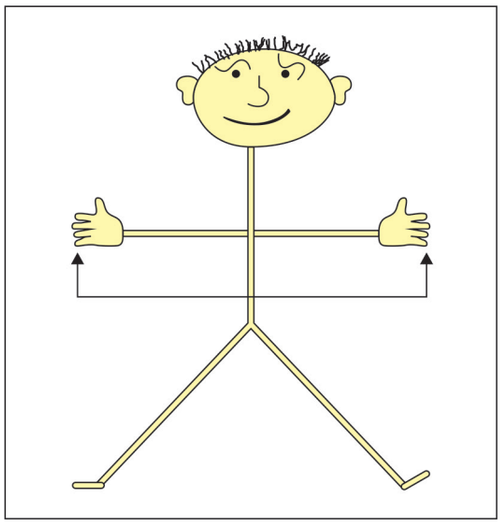

Fig. 2.5: Measurement of span
Courtesy: Line drawing by Master Aditya S

(Laurence-Moon-Biedl syndrome). Thin structure is seen in tuberculosis and anorexia nervosa.

Secondary Sexual Characteristics

Breast

- Breast tissue is very sensitive to oestrogen. Breast changes begin around 9 years of age and full development takes 5 years. It is unusual for no breast tissue to develop by 13 years of age.
- Breast development is absent or-ill developed in Turner's syndrome, hypopituitarism, pure gonadal dysgenesis, due to lack of oestrogen. Breast development is also poor in cretinism and congenital adrenal hyperplasia due to deficiency of oestrogen.
- Presence of galactorrhea would indicate the possibility of prolactinomas

Pubic and axillary hair

- Pubic and axillary hair development is called pubarche or adrenarche
- The pubic hair growth reflects the presence of oestrogen with the contributory effect from adrenal sex steroids, such as DHEA, DHEA-S and androstenedione which are weak androgenic substances.
- Absent pubic and axillary hair is seen in Turner's syndrome, hypopituitarism and pure gonadal dysgenesis due to lack of oestrogen.
- In testicular feminization, the axillary and pubic hair are absent due to deficiency of androgen binding receptors.

Hirsutism

Excessive hair growth with male pattern of distribution with or without virilisation is seen in PCOS, congenital adrenal hyperplasia, Cushing's disease, androgen producing tumours of ovary such as hilus cell tumour and arrhenoblastoma.

Somatic abnormalities

Somatic abnormalities are commonly seen in Turner's syndrome (Table 2.5) and 20% of Rokitansky-Kuster-Hauser syndrome.

Increased Carrying Angle (Cubitus Valgus) (>10–15°)

Carrying angle is the angle between the long axis of the humerus and the long axis of the ulna when the forearm is supinated. Carrying angle is 10–15° in extension and disappears in full flexion. If the carrying angle is increased, the condition of cubitus valgus results. If it is obliterated, the condition of cubitus varus results. In Turner's syndrome, the carrying angle is increased. The

Table 2.5: Somatic abnormalities associated with Turner's syndrome

Short stature	
Craniofacial abnormalities	Low set and malformed ears, low hairline, short webbed neck, epicanthal folds, high arched palate, abnormal teeth, micrognathia, short webbing of the neck (pterygium coli). In the webbing of the neck, the neck lies on the shoulder line and is better seen from behind (Fig. 2.6). Patients may have dental crowding or malocclusion
Auditory and visual defects	Ptosis, strabismus, amblyopia, red-green colour blindness, serous otitis media, hearing loss due to otosclerosis
Chest	Shield chest, widely placed nipple
Cardiovascular system	Coarctation of the aorta seen in 10% of cases, VSD, bicuspid aortic valve, high blood pressure which may be apparent even in childhood
Renal	Horseshoe kidney, unilateral renal aplasia
GI tract	Telangiectasis, GI bleeding due to intestinal vascular malformations, the incidence of Crohn disease and ulcerative colitis is also increased
Skin	Pigmented nevi, lymphoedema of hands and feet (Fig. 2.7), cystic hygroma, hypoplasia of the nail
Skeletal system	Cubitus valgus (increase in carrying angle), osteoporosis, short 4th and 5th metacarpal and metatarsal bones (Fig. 2.8) (Madelung deformities) of the wrist, polydactyly
Autoimmune diseases	Diabetes and thyroid dysfunction

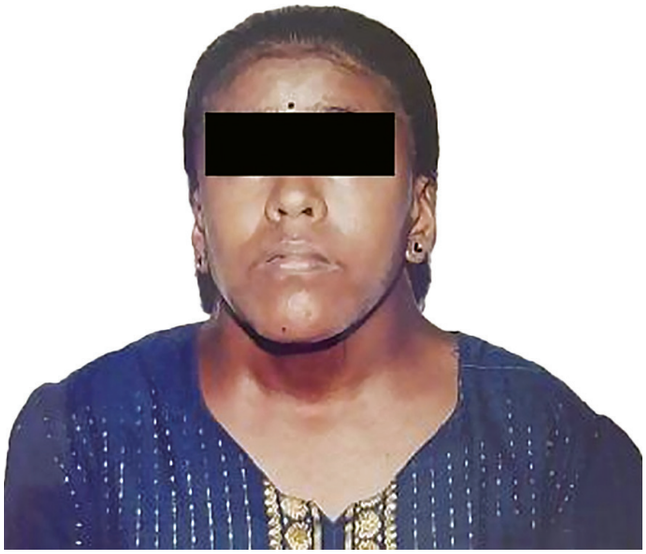

Fig. 2.6: Webbing of the neck in Turner syndrome

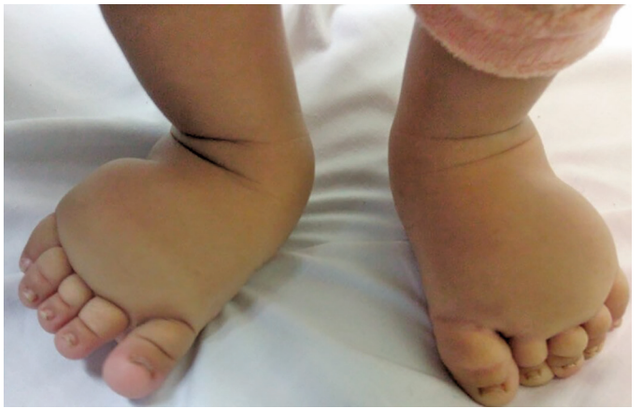

Fig. 2.7: Lymphoedema of feet in Turner syndrome
Courtesy: National Human Genome Research Institute

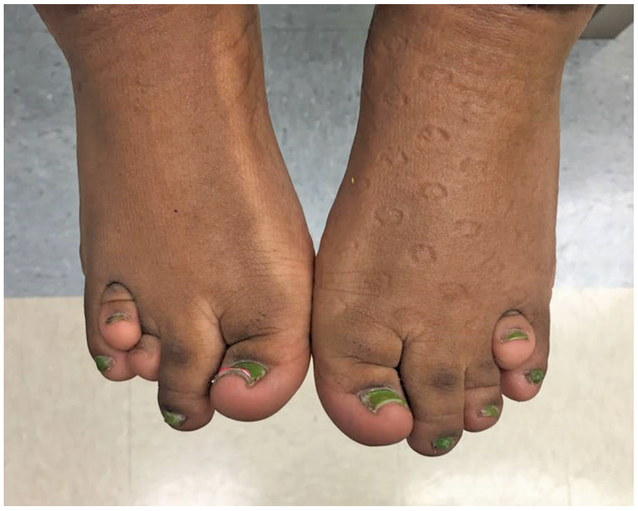

Fig. 2.8: Skeletal deformity of feet in Turner syndrome
Courtesy: Wikipedia

angle may also be distorted by fracture of lower end of humerus or rupture of the collateral ligament (Fig. 2.9).

Somatic abnormalities associated with MRKH syndrome

Commonly seen abnormalities in MRKH syndrome are scoliosis, cervical rib, pectus excavatum and Klippel-Feil syndrome (wedged vertebra, fused vertebrae, syndactyly, absent digits).

General examination

- On general examination, the neck is examined for cervical lymph node enlargement, thyroid enlargement, pigmentation, acne, and look for acanthosis nigricans.
- One should look for anaemia, signs of malnutrition which are commonly seen in tuberculosis, HIV/AIDS and chronic diseases. Adolescents with severe anaemia can present with delayed puberty.

Cardiovascular examination

Cardiovascular involvement may be seen in the following conditions:

- Turner's syndrome—VSD, coarctation of the aorta
- Laurence-Moon-Biedl syndrome—coarctation of aorta
- MRHK syndrome—congenital heart disease

Abdominal examination

- Abdominal striae in Cushing's disease
- Liver and spleen enlargement
- Lower abdominal mass may be due to haematometra caused by imperforate hymen, vaginal septum or displaced kidneys in müllerian agenesis
- In encysted tuberculosis ascites may be present
- Ovarian tumours

Examination of inguinal region

- Inguinal hernia may be seen in RHKS and testicular feminisation syndrome.
- Gonad may be palpable in the inguinal or labial region in testicular feminisation syndrome.

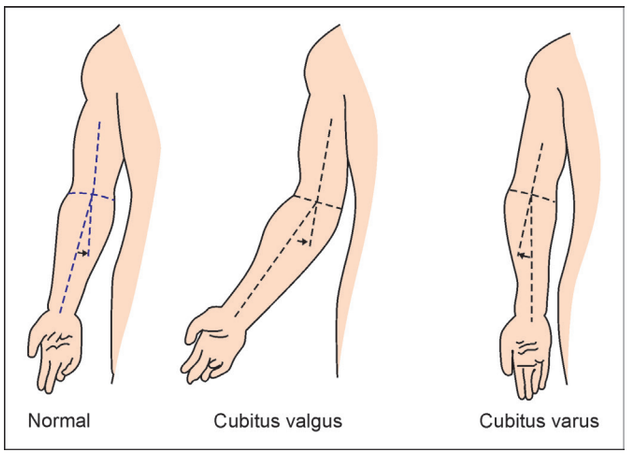

Fig. 2.9: Different degrees of carrying angle
Courtesy: Wikipedia

Local Examination of External Genitalia

- Enlarged clitoris is seen in congenital adrenal hyperplasia, Cushing's syndrome, cryptorchid male intersex and virilising male intersex.
- Ambiguous external genitalia are seen in cryptorchid male intersex and virilising male intersex and true hermaphrodite.
- Absence of vaginal opening is seen in congenital adrenal hyperplasia, vaginal agenesis (MRKHS), testicular feminization syndrome, and imperforate hymen.
- Bulging bluish membrane is seen in imperforate hymen.

Pelvic examination under anaesthesia

- Closure of the upper part of the vagina is seen in transverse vaginal septum and in agenesis of upper 1/3rd of vagina.
- Short vagina in testicular feminization syndrome and MRKH syndrome.

Rectal examination

Rectal examination may be helpful to assess the uterus, cervix and fornix details. Haematometra and haematocolpos can be made out easily by per rectal examination. However, with the advances in imaging studies, rectal examination is not usually carried out.

Investigations

As there are several causes for primary amenorrhoea, a wide spectrum of investigations are available for evaluating primary amenorrhoea. However, the specific investigations should be individualized depending upon the clinical findings.

General investigations

Routine urine investigations, complete haemogram, ESR, Mantoux, and X-ray chest are carried out to assess the general condition of the individual, and to diagnose anaemia and tuberculosis. Serum β-hCG may be carried out to rule out pregnancy if necessary.

Imaging Studies

A. *Ultrasonography*
   - Two-dimensional ultrasound examination is the initial investigation of choice as it is easily available. In the adolescent group, transabdominal USG is the preferred route. It is useful in diagnosing cases with outflow obstruction, to evaluate the ovarian morphology, to look for the presence or absence of uterus and to look for renal tract abnormalities such as horseshoe kidneys, unilateral or bilateral pelvic kidneys and unilateral renal aplasia.
   - Distended uterus and vagina with haematocolpos and haematometra may be diagnosed.
   - Presence of normal uterus and ovaries may indicate constitutional delay, PCOS, prolactinomas.
   - If the uterus is absent, uterovaginal agenesis and testicular feminization syndrome should be suspected.

B. *MRI of pelvis*

   Magnetic resonance imaging with its high soft tissue resolution allows accurate evaluation of uterine defects, rudimentary horns and presence of endometrial stripes in hypoplastic uterus. It is very useful for the anatomical evaluation of MRKH syndrome and is considered the gold standard for diagnosis of müllerian abnormalities.[8]

C. *Cranial MRI/CT brain*

   Cranial imaging would be required in the presence of symptoms such as headache, visual disturbances, or when a prolactinoma is suspected. Hypothalamopituitary causes such as congenital hydrocephalus, empty sella syndrome, tuberculoma, craniopharyngioma and prolactinomas can be diagnosed.

D. *Echocardiogram*

   Echocardiogram would be required to diagnose cardiac problems in cases of MRKH/Turner's syndrome/and Lawrence-Moon-Biedl syndrome.

E. *Intravenous pyelogram (IVP)*

   IVP would be rarely required because most of the renal tract anomalies can be diagnosed with ultrasonogram and MRI.

F. *X-ray of the long bones*

   All pubertal growth parameters correlate more closely with the bone age than with the chronological age and the bone age provides a useful marker of the degree of hypothalamic maturity. Therefore, in conditions where hypothalamic involvement is suspected, X-ray of the long bones (left wrist X-ray) is taken to assess the bone age and to see whether it corresponds to the chronological age. The skeletal maturity and chronological age may differ by as much as 2 years, and still may be within the normal limits.

Visual Field Assessment and Fundoscopy

- Papilloedema would indicate intracranial pathology.
- Myopia may be present due to retinitis pigmentosa seen in Laurence-Moon-Biedl syndrome.
- Lateral visual field defect may be present in prolactinomas.

Neurological Assessment

Neurological assessment would be required in cases presenting with anosmia to study the olfactory nerve function (Kallmann's syndrome).

Biochemical Investigations

a. Free thyroxine and thyroid stimulating hormone estimation: Severe hypothyroidism can present with

primary amenorrhoea and hypothyroidism may be associated with Turner's syndrome.

b. Serum prolactin levels: May be raised in chronic hypothyroidism, prolactinomas or may indicate pituitary stalk compression leading to primary amenorrhoea.

c. Oral glucose tolerance test: Diabetes may be associated with Turner's syndrome, Cushing's syndrome and Lawrence-Moon-Biedl syndrome.

d. Estimation of serum FSH and LH levels

- High gonadotrophin levels: Gonadotrophin levels are elevated in conditions where there is absence or failure of ovarian function. Due to absence of oestrogen there is no negative feedback on the pituitary which continues to secrete gonadotrophins leading to hypergonadotrophic hypogonadism.

  Hypergonadotrophic hypogonadism is seen in Turner, Turner mosaic, pure gonadal dysgenesis, premature ovarian failure, resistant ovary syndrome and destruction of ovary due to infections, autoimmune disease, radiotherapy or chemotherapy. Increased LH/FSH ratio is also seen in pre-pubertal PCOS.
- Low gonadotrophin levels: Low gonadotrophin levels are seen when the pathology is either in the pituitary or in the hypothalamus. Pan hypopituitarism is seen in congenital hydrocephalus, trauma, empty sella syndrome, craniopharyngioma which can destroy the pituitary or the pituitary stalk. There are also hypothalamic causes such as Isolated GnRH deficiency, olfactogenital syndrome, Lawrence-Moon-Biedl syndrome and Prader-Willi syndrome where the pituitary is normal, but there is no stimulation from the hypothalamus.

  Gonadotrophin levels are also low in conditions such as weight loss, anorexia nervosa and hyperprolactinaemia.

e. GnRH stimulation test: In order to differentiate the hypothalamic causes from pituitary causes, GnRH stimulation test can be performed. If the low levels of gonadotrophins are due to hypothalamic causes, administration of GnRH will increase the pituitary gonadotrophin levels as the pituitary is normal.

f. Estimation of adrenal steroids: 17-OH progesterone, dehydroepiandrosterone sulphate and androstenedione. The levels are raised in congenital adrenal hyperplasia and adrenal tumours, Cushing's disease, PCOS and arrhenoblastomas.

g. Adrenocorticotrophic hormone stimulation test may be required to detect steroid synthesis defect such as congenital adrenal hyperplasia.

h. Dexamethasone suppression test: When the adrenal steroids are suppressed with dexamethasone it indicates adrenal hyperplasia. In adrenal tumours no suppression occurs.

Genetic Studies

Karyotyping is the *Gold Standard* method to diagnose genetic problems. Identification of Barr body is not used any more.

Examination Under Anaesthesia (EUA) and Laparoscopy

With the availability of imaging studies, especially MRI, a thorough anatomical evaluation of the pelvis is possible and is the gold standard for evaluating MRKH syndrome. However, in settings where MRI is not feasible, EUA and laparoscopy are useful for the evaluation of MRKH syndrome, tuberculosis or when there is heterosexual development.

Hysteroscopy

When end organ failure due to tuberculosis is a possible diagnosis, hysteroscopy would be required to diagnose intrauterine adhesions and to collect samples for investigations.

STEPWISE EVALUATION OF PRIMARY AMENORRHOEA

Though the causes of primary amenorrhoea are classified based on the site of pathology, for practical purposes and for clinical evaluation, cases are divided into three groups based on the secondary sexual characteristics.

I. Those presenting with normal secondary sexual characteristics.
II. Those presenting with absent secondary sexual characteristics.
III. Those presenting with heterosexual development.

EVALUATION OF PRIMARY AMENORRHOEA WITH NORMAL SECONDARY SEXUAL CHARACTERISTICS

Presence of normal secondary sexual development is an indication of normal hypothalamopituitary ovarian function. In these cases the causes may be constitutional delay, prolactinomas, PCOS, non-responsive endometrium as in tuberculosis, müllerian agenesis or due to outflow obstruction. Testicular feminization also presents with normal breast development (Fig. 2.10).

- In the evaluation of cases with normal secondary sexual characteristics, the initial evaluation is by USG of pelvis to look for the presence or absence of uterus.
- If the uterus is absent, karyotyping is carried out to differentiate between müllerian agenesis and testicular feminization syndrome.
- If the uterus is present, USG may show evidence of outflow obstruction.
- In the presence of normal anatomy of the genital tract, do a progesterone challenge test with tablet norethisterone 5 mg twice daily for one week.
- If there is withdrawal bleeding following progesterone within 10 days, it indicates that the primary amenorrhoea may be due to hypothyroidism, prolactinomas, PCOS,

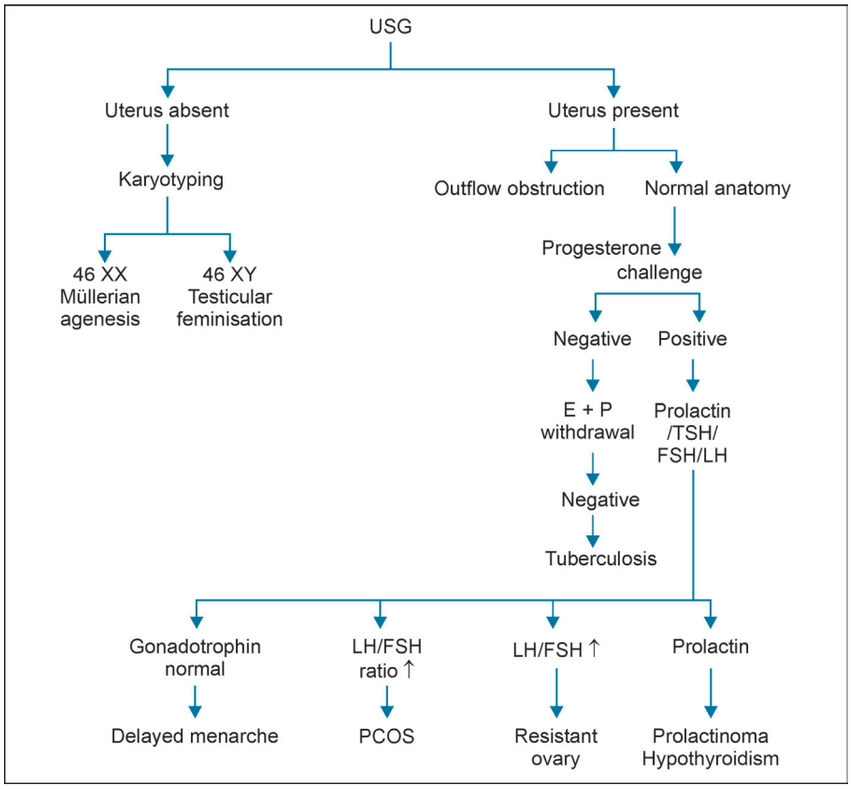

Fig. 2.10: Primary amenorrhoea with normal secondary sexual characteristics

resistant ovaries or constitutional delay. Therefore, estimate the FSH/LH/prolactin and TSH levels to differentiate the above conditions.

- In the absence of withdrawal with progesterone, the E + P challenge is given with combined OC pills for 10 days. Absence of withdrawal with E + P will confirm the diagnosis of non-responsive endometrium as seen in tuberculosis.
- Occasionally, PCOS with elevated androgen levels may not show withdrawal with progesterone challenge (due to androgen having an antagonistic effect on oestrogen), but will show withdrawal with E + P challenge.

CONDITIONS CAUSING PRIMARY AMENORRHOEA WITH NORMAL SECONDARY SEXUAL CHARACTERISTICS

Outflow obstruction may be caused by imperforate hymen, transverse vaginal septum, vaginal atresia with absent cervix or müllerian agenesis.

Depending upon the level of obstruction the patient develops haematoclpos and/or haematometra. The condition is also called cryptomenorrhoea. The collection of blood which distends the uterus can also cause haematosalpinx and depending upon the duration of obstruction may result in pelvic/ovarian endometriosis. Since the development of the müllerian system and urinary tract are closely associated, co-existent anomalies of the urinary system is a common finding. MRI is the investigation of choice to ascertain the level and the type of pathology.

Imperforate Hymen

- Imperforate hymen is reported in 1 in 1,000 women.
- The girl presents with cyclical lower abdominal pain, usually between the ages of 14 and 16.
- It can also present with difficulty in defaecation and retention of urine due to the pressure effect caused by haematocolpos and haematometra formation.
- Can also present with abdominal mass, as the uterus can be lifted up into the abdomen by the haematocolpos.
- Local examination will reveal bluish bulging membrane at the introitus (Fig. 2.11).

Transverse Vaginal Septum

Transverse vaginal septum is reported in 1 in 80,000 women. The patient presents with the same history as in imperforate hymen. The transverse vaginal septum may be seen at different levels in the vagina; lower 1/3rd, middle 1/3rd or upper third of vagina. On examination abdominal swelling may be palpated due to haematometra. On local examination, hymenal remnant will be seen, bulging membrane will be seen, but is not blue in colour due to high level of the septum. However, rectal examination will reveal the presence of

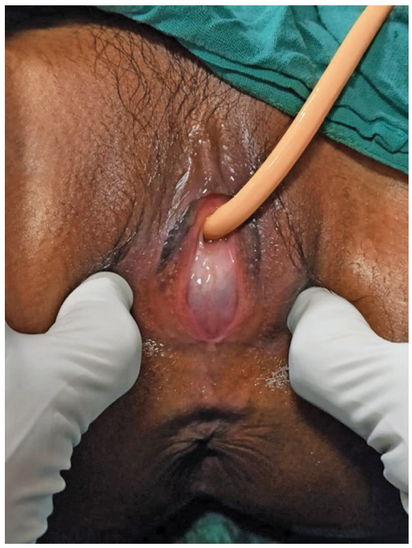

Fig. 2.11: Bluish bulging membrane at the introitus due to imperforate hymen

haematocolpos which bulges into the rectum. MRI is the best imaging modality to identify the level of obstruction and based on the level of obstruction the treatment can be planned. At the time of surgery, a wide bore needle can be passed to decide the depth at which the septum is situated. If the level of obstruction is low, excision of the septum and drainage can be done from the vaginal route. If the septum is in the upper 1/3rd of the vagina, abdominal vaginal approach may be required.

Absent Vagina and Functioning Uterus

There is partial agenesis or annular constriction of upper parts of vagina and the cervix is usually absent. Because of the functioning uterus, there is haematometra, haematosalpinx and there can be haemoperitoneum as well. On rectal examination, because of the absence of haematocolpos, there is no bulge into the rectal mucosa. As the cervix is absent, connecting the uterus to the vagina is not possible, and the conventional treatment is hysterectomy with reconstruction of vagina from below.

Absent Vagina and Non-functioning Uterus (Müllerian Agenesis/Mayer-Rokitansky-Kuster-Hauser Syndrome: MRKH Syndrome)

Mayer-Rokitansky-Küster-Hauser syndrome results from agenesis or hypoplasia of the müllerian ducts (paramesonephric ducts). The individual has normal female karyotype 46, XX, normal gonads and has a normal female development of secondary sexual characteristics and the phenotype is female. Because of the müllerian agenesis, there is congenital absence of upper two-thirds of the vagina and the uterus is either absent or rudimentary with solid müllerian knobs connected by a fibromuscular band. By MRI studies, rudimentary müllerian structures are found in 90% of patients with müllerian agenesis. In 7–10% of these cases within the rudimentary uterus, active endometrial tissue may be present and can cause cyclical abdominal pain due to the associated haematometra. The cyclical abdominal pain may necessitate removal of the rudimentary horn. Presence of functional endometrial tissue can also lead to haematosalpinx and endometriosis.

The incidence of congenital absence of the vagina is 1 per 4000–5000 female births.[10] MRKH syndrome is a congenital disorder that is present at birth but may remain undiagnosed until adolescence or early adulthood. MRKH syndrome is one of the common causes of primary amenorrhea. In Parul Shah's study among the 60 cases of primary amenorrhoea, 22 were due to müllerian agenesis (nearly 30%).[11] In author's own study among the 42 cases of primary amenorrhoea, 13 (31.7%) were due to müllerian agenesis.[12] In recent years, MRKH syndrome is classified into three types.[13]

- Typical (type I): Isolated symmetrical uterovaginal aplasia or hypoplasia.
- Atypical (type II) asymmetrical uterovaginal aplasia or hypoplasia or absence of one or both fallopian tubes, and malformation in the ovaries and/or the renal system.
- MURCS (type III) (müllerian duct aplasia, renal dysplasia and cervical somite anomalies) syndrome. Here, along with uterovaginal agenesis, in nearly 53% of cases, there is associated malformation of the heart, skeletal and renal system.

Müllerian agenesis is associated with urogenital malformations such as unilateral renal agenesis, pelvic kidney, horseshoe kidney, hydronephrosis, ectopic kidney and ureteral duplication and are seen in 30–40% of cases.[14] Skeletal abnormalities are reported in 12–20% of women presenting with fused vertebrae, wedged vertebrae, radial aplasia, absent thumb, scoliosis, and pectus excavatum (Klippel-Feil syndrome).[15] Skeletal malformations are due to alterations of the blastomeres of the lower cervical and upper thoracic somites.[16] Auditory defects are reported in 10–25% of cases.[10] Occasionally, it can be associated with major congenital heart disease like Fallot tetralogy/truncus arteriosus and inguinal hernia.

Clinical Presentation

- The patient presents with normal pubertal changes with breast, pubic and axillary hair development, but absent menstruation.
- In the presence of a functioning, non-communicating rudimentary horn, the patient presents with cyclical abdominal pain, unilateral or more on the affected side.

- As the ovarian function is normal, the patient experiences normal premenstrual symptoms. Other common presentations are infertility and dyspareunia.
- The patient may experience voiding difficulties, urinary incontinence, or recurrent urinary tract infections (UTIs) due to co-existent renal involvement or due to the pressure effect.[17]

Examination Findings

- Normal secondary female sexual characteristics are present
- Height is normal
- Examination of the external genitalia is normal with normal vulva, labia majora, labia minora, and clitoris.
- The vagina is either absent or short with a blind pouch.
- General examination may reveal skeletal and other somatic abnormalities.

Differential Diagnosis

Müllerian agenesis must be differentiated from complete androgen insensitivity because the vagina may be absent or short in both disorders. The other conditions to be considered are transverse vaginal septum and imperforate hymen.

Investigations

- Chromosomal analysis is important in order to differentiate müllerian agenesis from androgen insensitivity syndrome. Individuals with complete androgen insensitivity syndrome (testicular feminization) have female external genitalia but a 46, XY karyotype, whereas in müllerian agenesis the chromosomal pattern is 46, XX.
- Because of the presence of secondary sexual characteristics, an assay of FSH/LH is not required.
- While awaiting karyotyping results, testosterone levels can be assayed and are in the normal female range in müllerian agenesis, whereas it is raised in androgen insensitivity syndrome.
- USG is useful to identify the level of vaginal aplasia, to identify uterine duplications, the presence of functioning rudimentary horns, and the presence of endometriomas. It also allows assessment of the kidneys and bladder for abnormalities.
- *MRI is the gold standard technique for evaluating congenital anomalies of the uterus.* It provides excellent images of superficial and deep tissue planes. It can delineate the cervix. Suspected vertebral anomalies are easily imaged by MRI. Magnetic resonance urography (MRU) is an excellent imaging modality for the visualisation of the reproductive and urinary anatomy.
- *Intravenous or retrograde pyelography may be required to assess the renal structure.*
- Laparoscopy is not necessary unless associated with cyclical abdominal pain to rule out uncanalised myometrial tissue or non-communicating rudimentary horn. The rudimentary horn can be removed through laparoscopy. Laparoscopy is also useful when pelvic endometriosis is suspected.

Aetiology of Müllerian Agenesis

Though MRKH syndrome is generally considered to be a sporadic condition, familial clustering is reported with increasing frequency. The aetiology of müllerian agenesis remains unclear, however, many theories have been suggested:[18]

1. Development of the vagina takes place between 37 and 41 days of gestation and teratogenic exposure during this period has been suggested as a possible aetiology.
2. Single gene mutation with familial clustering has been observed. These are autosomal dominant genes transmitted by male relatives.
3. Other proposed theories are inappropriate production of müllerian regression factor, absence or deficiency of estrogen receptors in the lower müllerian duct and mesenchymal inductive defect.
4. *WNT4* genes are associated with gonadal differentiation and müllerian duct development. Mutation of the gene has been reported in girls with müllerian aplasia.[15]

Management

There should be a multidisciplinary approach in the management of these patients involving an endocrinologist, an adolescent gynaecologist, a geneticist, an orthopaedic specialist, a urologist and a psychiatrist. The treatment should aim at:

1. Creating a neovagina for sexual function. The creation of a neovagina may be postponed until the woman is considering marriage and become sexually active.
2. In the presence of functioning uterus and cervical tissue, the uterus can be implanted in the vagina following vaginoplasty concurrently or in a second stage surgery.
3. Options for having children in the future should be discussed with patients, which may help them to cope with the diagnosis and its implications.
4. The options for pregnancy include adoption and gestational surrogacy. As the ovaries are normal, by using the woman's own eggs, genetically related off spring can be produced using *in vitro* fertilisation and surrogacy. As transvaginal ovum retrieval will be difficult, transabdominal or laparoscopic egg retrieval may be necessary.[19]
5. There is interest in the possibility of uterine transplantation for women with uterine factor infertility, including those with MRKH syndrome.[20]

Surgical Management in MRKH Syndrome

The goal of surgical management is to provide unscarred vagina for sexual function as well as to prevent

endometriosis and its related morbidity and ovarian function impairment.

Creating a Neovagina

Creating a neovagina either by nonsurgical or surgical method should wait until the patient is emotionally mature and understands the implications of the surgery.

Use of Vaginal Dilators

Use of vaginal dilators to dilate the vagina is a non-invasive way of creating the neovagina and should be the first line of approach (Fig. 2.12). It is safe to use, the patient learns the technique easily and is more cost effective than surgery. In a well motivated patients, > 90% of patients will be able to achieve anatomic and functional ability with primary vaginal dilatation. In the initial part of teaching the individual, it should be carried out in a supportive setting with close monitoring. Using a mirror, the individual is explained the anatomy of the external genitalia and taught to correctly locate the apex of the vagina. She should be instructed to place progressive dilators on the vaginal apex for 10–30 min one to two times a day.

Frank Procedure

It is another non-surgical procedure where the patient creates a neovagina by applying progressive pressure to the perineum, using a bicycle-seat stool which holds a dilator in place. In patients with absent vagina, the compliance is poor because of the discomfort.

Surgical Creation of Neovagina

Surgery is reserved for those individuals in whom use of vaginal dilators was not successful. The aim of surgery to create a vagina suitable for penetrative intercourse. Following surgery, there should be ongoing vaginal dilatation/sexual intercourse to maintain adequate the depth and width of the vagina. Surgery is often performed in late adolescence or young adulthood when the patient is mature enough to understand the procedure as well as to follow postoperative vaginal dilatation. Vaginal reconstructive procedure may be associated with complications such as bladder/rectal perforation and graft rejection.[20]

Fig. 2.12: Vaginal dilators

McIndoe Technique

In this technique, a space is created between the urethra and rectum by blunt dissection. A split-thickness skin graft is taken from the thigh and covered over a cylindrical stent which is placed into the created space and fixed to the cut edges of the skin incision. The labia majora are approximated over the stent to hold it in place. The stent is removed 1 week later, the neovagina is irrigated, and any areas of granulation tissue are cauterized with silver nitrate sticks. The patient is advised to use the mould or dilator in the neovagina every day for 3 months. Ideally, the procedure should be performed before the initiation of sexual function to avoid secondary closure of the vagina. These women also need HPV vaccination to protect against vulval and vaginal cancer and genital warts.

Williams Vaginoplasty

In this procedure, the labia are used to create a neovagina. This simple procedure does not damage the urethra or rectum, however, the neovagina has a physiologically abnormal angle.

Other techniques use flap procedures using gracilis muscle, or by using isolated segment of bowel for creating the vagina. Laparoscopic procedures are also available. Complications of these procedures include skin scarring at the donor graft site, postoperative rectovaginal and urethrovaginal fistulas, infection and vaginal discharge, vaginal stenosis and dyspareunia.

XY Female—Complete Androgen Insensitivity Syndrome—Testicular Feminization Syndrome

Complete androgen insensitivity is rare, having an incidence as low as 1 in 60,000, but it accounts for approximately 5% of all cases of primary amenorrhoea.[21] This condition is due to the deficiency of cytosol androgen receptors in the target organ, which are necessary for the action of testosterone. This defect is genetically determined and inherited by an X-linked recessive gene. There is mutation in the androgen receptor gene localized to the long arm of the X chromosome (Xq11–13). It can run in families where maternal aunt and sisters can be affected. The genetic makeup of the individual is 46, XY and the gonad develops into normal testicular tissue with immature germ cells, Sertoli cells and prominent Leydig cells. However, testes are situated in the inguinal canal, labia majora or in the ovarian fossa. There may be associated inguinal hernia in these cases. The testes are hormonally competent and they produce both testosterone and anti-müllerian factor. In the presence of anti-müllerian hormone, the müllerian structures do not develop and there is no uterus and the vagina is short. Though the testosterone production is normal, because of the receptor deficiency, Wolffian structures do not develop and there are no internal genital organs. Because of the absence of receptors, the external genitalia

also develop into female structures. Plasma testosterone levels are slightly higher than normal males. The brain is also insensitive to the circulating androgens, as a result psychologically also the individual develops into a female. Adult women with complete androgen insensitivity syndrome are generally taller than women without the syndrome, but are on average shorter than the male population.

In the periphery there is adequate breast development, because the normal adrenal and testicular androgens are converted to oestrogen which results in the development of the breast. Because of the absence of androgen binding receptors, the hair follicles do not respond to the androgens resulting in absent or scanty axillary and pubic hair. This condition has to be differentiated from müllerian agenesis by karyotyping. The chromosomal pattern of testicular feminization syndrome is 46, XY whereas that of müllerian agenesis is 46, XX. Alternatively, the presence of a Y chromosome can be confirmed by fluorescence *in situ* hybridization (FISH) probes for the *SRY* region of the Y chromosome. These methods take a shorter time than conventional karyotypes. Mutation analysis of androgen receptor gene is also available which detects 95% of mutations for complete insensitivity syndrome.

Management

Management of androgen insensitivity syndrome should address the sexual function, psychological issues related to diagnosis and gonadectomy and subsequent hormone replacement, creation a neovagina, and genetic counseling.

- These individuals are reared as females.
- As the gonads are in ectopic sites they are vulnerable to undergo malignant changes especially dysgerminomas. Common malignancies associated with testicular feminization syndrome are dysgerminomas, seminomas, and gonadoblastomas. The reported incidence of gonadal malignancy in this condition is 22%, but it rarely occurs before the age of 20 years. Gonadectomy is mandatory in this condition, however, it has to be postponed until the development of the secondary sexual characteristics and allowing time for the epiphyseal closure to attain normal adult height. Normally, gonadectomy is carried out between the ages of 16 and 18 years.
- After gonadectomy, hormone therapy with oestrogen will be required to prevent osteoporosis. Hormone therapy is initiated with low dose oestrogen initially, gradually increased to adult dose. In order to assess the effectiveness of hormone therapy, bone mineral density is measured every two years.
- Creating a neovagina for sexual function is important either by vaginoplasty or by using vaginal dilators. Vaginal dilators are an effective first-line treatment. These procedures should be delayed until the individual is sufficiently mature to understand the treatment as well as prepared for sexual activity.
- Psychological support is very important as they are reared as females.
- In any newborn female child born with inguinal hernia, one should suspect testicular feminization syndrome and karyotyping should be undertaken.[22] If diagnosed in early childhood, the management options are:
  1. Early gonadectomy with puberty induction. The main advantages are the child is unaware of the issues and the risk of malignancy is prevented. Puberty is induced with 2 μg of ethinyl oestradiol at the age of 11 years.
  2. Gonadectomy can be delayed until early adulthood between 16 and 18 years allowing time for normal growth spurt and breast development.
- Loss of fertility is a major issue for many women with complete androgen insensitivity syndrome. Individuals can adopt or might choose to use donor oocytes and a surrogate mother with their partner's sperm to achieve a pregnancy.

Prevention

- Parents need genetic counseling to understand the risk of recurrence (25% for each subsequent pregnancy), as well as to identify other potential carriers.
- In any newborn female child born with inguinal hernia should be evaluated for testicular feminization syndrome and karyotyping should be undertaken.

Endometrial Defects and Non-receptive Endometrium

In girls where the endometrium has been destroyed by tuberculosis, they do not respond to the normal hormonal stimulation resulting in primary amenorrhoea. Diagnosis is made by the presence of normal secondary sexual characteristics indicating normal functioning of the hypothalamo-pituitary-ovarian axis and the development normal internal and external genital organs. Hysteroscopy and hysterosalpingogram may show evidence of intrauterine adhesions and marked narrowing of the endometrial cavity due to fibrosis. In these patients progesterone challenge test and estrogen + progesterone challenge test will be negative. Very rarely, oestrogen receptor deficiency has been reported in the endometrium leading to non-responsive endometrium.

Constitutional Delay

In these individuals, the pubertal activation and the maturation of the hypothalamopituitary or gonadal axis and pulsatile release of GnRH are delayed leading to delayed physiological development: height, genital development and bone maturation. In spite of the development of secondary sexual characteristics, menarche is delayed. History of delayed menarche can be elicited from the sibling and parents and genetic

influence may play a role. These individuals have normal secondary sexual development and there is no anatomical defect and their endocrine profile, the gonadotrophins, adrenal and gonadal sex steroids are appropriate for their developmental age (bone maturation). There is positive response to progesterone challenge test and GnRH stimulation increases the LH levels. Reassurance in the mainstay of treatment and the delay is time limited. No hormone therapy is required.

Resistant Ovary Syndrome (Savage Syndrome)

In these individuals due to the gonadotrophin receptor deficiency, there is insensitivity to gonadotrophins, as a result complete ovarian follicular maturation does not occur. They are normally appearing 46, XX adolescents or adult females showing normal height and secondary sexual characteristics. USG may show varying number of follicles and the gonadotrophin levels are increased. They are managed with high doses of gonadotrophins to trigger follicular maturation and menstruation. If this fails, hormone therapy is an option for menstrual function and hormone replacement.

Prolactinomas, Adolescent PCOS and Hypothalamic Causes

Hyperprolactinemia due to prolactinomas of pituitary, early onset PCOS, severe psychological stress, weight loss due to severe exercise, anorexia and chronic illness can cause hypothalamic dysfunction. These conditions most often present as secondary amenorrhoea, but, occasionally can present as primary amenorrhea. These individuals present with normal secondary sexual characteristics with functioning uterus. Treatment of the underlying cause usually reverts the dysfunction, culminating in menarche.

Other Causes of Primary Amenorrhoea with Normal Secondary Sexual Characteristics

Severe malnutrition, obesity, chronic nephritis and cirrhosis can present with primary amenorrhoea due to impairment of metabolism of oestrogen. HIV infection should be considered in those who are severely malnourished. If diagnosed, regular menstrual cycles can be restored with weight gain using highly active antiretroviral therapy (HAART).

PRIMARY AMENORRHOEA WITH ABSENT SECONDARY SEXUAL CHARACTERISTICS

The absence of secondary sexual characteristics indicates that there is no oestrogen secretion from the ovaries. This may be due to:

- In primary hypogonadism (primary ovarian failure) due to some pathology in the ovary, the ovaries do not secrete adequate amounts of oestrogen which results in sexual infantilism. Because of the absence of negative feedback by the oestrogens, there is increased secretion of gonadotrophins. This scenario is called hypergonadotrophic hypogonadism.
- Secondary hypogonadism (gonadotrophin deficiency): The ovaries are normal, but are not stimulated due to lack of endogenous gonadotrophin secretion, due to defects at the hypothalamopituitary level. There may be isolated GnRH deficiency or the secretion of several other hormones such as growth hormone, thyroid hormone is also affected. This situation is called hypogonadotrophic hypogonadism. The deficiency of gonadotrophins may be idiopathic or secondary to a tumour of the pituitary or hypothalamus.

Evaluation of sexual infantilism

- Whenever sexual infantilism with absent secondary sexual characteristics is encountered, the initial evaluation should be accurate measurement of the height of the individual.
- Short stature with sexual infantilism may be due to Turner, Turner's mosaic or pan hypopituitarism.
- Girls with normal height with sexual infantilism may be due to pure gonadal agenesis (dysgenesis), ovarian failure due to various reasons and isolated GnRH deficiency.
- In order to find out the exact cause of primary amenorrhoea in these individuals, serum FSH, LH and karyotyping should be carried out. As shown in Fig. 2.13, algorithm should be followed to arrive at the cause of primary amenorrhoea due to sexual infantilism.

Conditions Causing Primary Amenorrhoea with Absent Secondary Sexual Characteristics

Turner Syndrome

In 1938, Henry Turner first described Turner syndrome, which is one of the most common chromosomal abnormalities. This syndrome is characterized by absence of one X chromosome and the karyotyping is 45, XO. In the absence of two sex chromosomes, gonadal development does not occur, and it appears as a band of fibrous tissue called streak gonad. Normal gonadal development occurs until 20 weeks of gestation after which there is failure of development of primordial follicles and at birth there are no oocytes.[23]

Genetics of turner syndrome

- The classic Turner with monosomy with 45, XO is seen in 45–50% of cases due to non-disjunction during meiosis (Table 2.6).
- Turner mosaics with 45, XO/46, XX, 45, XO/46, XY is seen in nearly 30% of cases. Here, one of the sex chromosomes is lost in some of the cell lines of body and these cells have only 45, XO chromosomes. Whereas, the other cells have normal chromosomal pattern with 46, XX or 46, XY. The clinical features

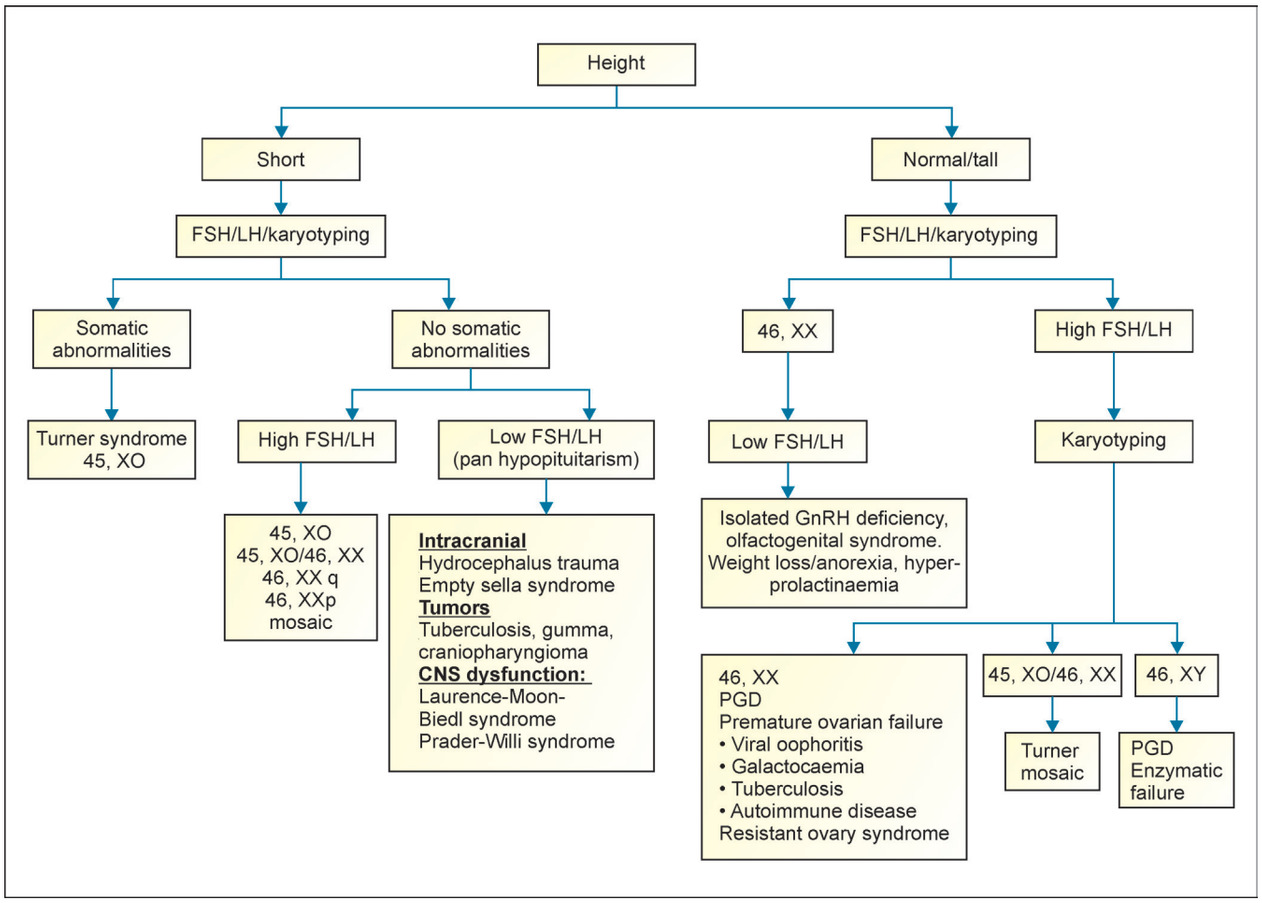

Fig. 2.13: Evaluation of primary amenorrhoea with absent secondary sexual characteristics

Table 2.6: Genetics of Turner syndrome

Disease	*Karyotyping*	*Frequency%*	*Mechanism*
Monosomy 45, XO Classic Turner	45, XO	45–50%	Non-disjunction during meiosis
Turner mosaic	45, XO/46, XX, 45, XO/46, XY	30%	Occurs during early mitotic cell division
Isochromosomes	X_P–X_Q	10%	Occurs during anaphase
Deletions, translocations		10%	Occurs during meiosis
Ring chromosomes		2–5%	Loss of centromere causing ring to form

of Turner syndrome correlate with the relative percentage of 45, X cells within the body.

- In 46 isochromosomes X_P–X_Q rather than complete loss, there are structural abnormalities with deletion involving the short and long arm of the X chromosome. There could also be formation of ring chromosomes.
- In some of these variants, few oocytes may be retained until puberty, may establish normal periods initially, later on go in for premature ovarian failure.
- In Turner mosaic with Y chromosome, there is risk of gonadal malignancy and the gonads should be removed.

Turner syndrome is one of the most common chromosomal abnormalities, occurring in approximately 1 in 2000 live-born female infants.[24] As many as 15% of spontaneous abortions have a 45, X karyotype and 99% of conceptions with 45, XO karyotypes spontaneously abort. As the gonad is not developed, the individual develops into a female with sexual infantilism. The müllerian structures and the external genitalia are of a female (Fig. 2.14). In the absence of oestrogen, there is no negative feedback on the H-P-O axis, thereby increasing the gonadotrophin level giving rise to hypergonadotrophic hypogonadism.

Turner syndrome

Pedigree analysis has shown that Turner's syndrome can be a heredo-familial disorder. The patient with Turner's

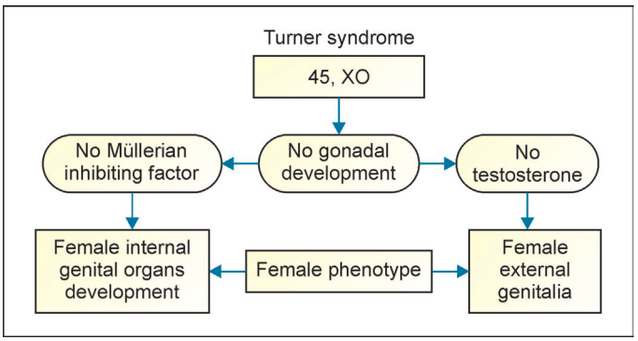

Fig. 2.14: Female development in Turner syndrome

syndrome and/or her parents may suffer from diabetes/hypothyroidism or other autoimmune disorders. To explain this, various types of hypothesis have been described.

a. The presence of autoantibody in one of the parents may directly affect the development of the germ cells.
b. Some fault in genetic stability which could lead both to the occurrence of autoantibodies and to the existence of abnormal gametes.[25]

Clinical Features of Turner Syndrome

Sexual infantilism

Individuals with Turner syndrome present with sexual infantilism. They have female phenotype, and do not exhibit pubertal changes, there is no breast, pubic and axillary hair development and the internal and external genital organs are infantile. They present with primary amenorrhoea. If the presentation is secondary amenorrhoea they also present with infertility. Both LH and FSH are elevated to menopausal levels.

Short stature

Almost all individuals with Turner syndrome have short stature. They often have normal height up to 5 years of age, then have a slow growth rate and the normal pubertal growth spurt does not occur. The height is usually <140–145 cm and this is due to the loss of one copy of the SHOX gene on the X chromosome which is important for long bone growth. In Turner syndrome, the arm span is > the height by at least 1–1.5 inches.

Endocrine system involvement

The patient with Turner syndrome and/or her parents may suffer from diabetes hypothyroidism or other autoimmune disorders. Somatic abnormalities associated with Turner's syndrome represented in Table 2.5.

Diagnosis

Turner syndrome is often diagnosed in early childhood because of the well-known phenotypic characteristics (short stature, webbed neck and low hairline) and the confirmation is done by karyotyping.

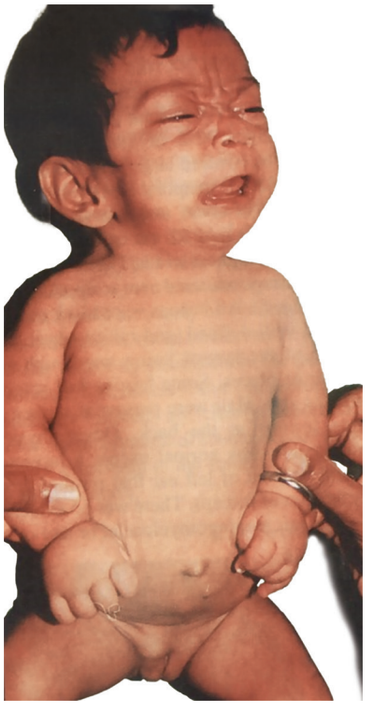

Fig. 2.15: Female child showing webbing of the neck, widely placed nipples and swollen hand in Turner syndrome
Courtesy: IAP Journal 2001

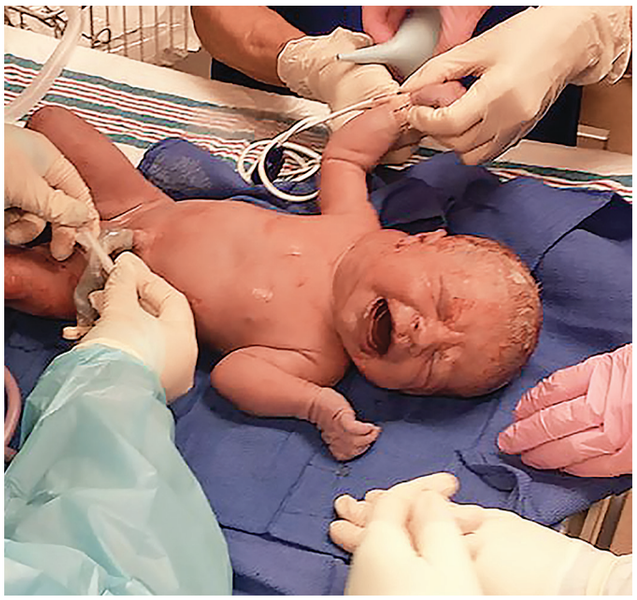

Fig. 2.16: Child born with cystic hygroma in Turner syndrome
Courtesy: Wikipedia

Clinical features at birth

Any female child born with swollen hands and feet due to lymphedema (Fig. 2.15), cystic hygroma (Fig. 2.16) or cardiac defects, one should suspect Turner syndrome and karyotyping should be carried out.

Differential Diagnosis

Noonan syndrome

This condition is caused by changes in one of the autosomal dominant genes. They present with unusual facial characteristics, they are short statured with webbing of the neck, heart defects, renal disorders, bleeding problems, developmental delays, and malformations of the bones of the rib cage. But they have normal karyotyping.

Investigations

Karyotyping

A standard 30-cell karyotype analysis is required for the diagnosis of Turner syndrome, and to exclude mosaicism. Diagnosis is confirmed by the presence of a 45, X cell line or a cell line with deletion of the short arm of the X chromosome (Xp deletion). Patients with Turner syndrome should be investigated for the presence of Y chromosomal material using a Y-centromeric probe. Patients with 45,X/46,XY mosaicism may have mixed gonadal dysgenesis and are at a high risk for gonadoblastoma. Patients with ring chromosomes or fragments of chromosomes should also be examined for Y chromosomal material. Because of the high risk of gonadoblastoma, these patients require prophylactic gonadectomy.[26]

Biochemical Investigations

Gonadotrophins

Both LH and FSH are elevated to menopausal levels after the age of 10 years. After confirming the diagnosis, the following investigations should be carried out periodically, as these patients suffer from various chronic medical conditions.

Thyroid function tests

Because of the high prevalence of hypothyroidism in Turner syndrome, thyroid function tests should be done at diagnosis and TSH measurements repeated every 1–2 years or if symptoms develop, because hypothyroidism may develop at a later age.

Glucose metabolism

Screening for diabetes mellitus should be carried out periodically for the patient as well as for the other family members.

Renal studies

Renal studies with USG, annual urine cultures and measurement of BUN and creatinine levels are recommended for those patients with renal abnormalities. Girls with horseshoe kidneys have an increased risk of *Wilms tumour* and should have renal ultrasound examinations every 4–6 months until the age 8 years and every 6–12 months thereafter.

Cardiovascular studies

Echocardiography and/or MRI of the heart and aorta are carried out at diagnosis. Because of the high incidence of coarctation of aorta, patients are evaluated with four limb blood pressure measurements. *Hypoplastic left heart, coarctation of the aorta,* bicuspid aortic valve, and aortic dissection are the cardiovascular malformations associated with Turner syndrome. All individuals should have an initial evaluation and periodic follow-up with a cardiologist. Evaluation prior to initiation of estrogen therapy or assisted reproduction is strongly recommended.[27] Patients with significant anomalies should have subacute bacterial endocarditis prophylaxis. Because of the risks of aortic root dilatation and mortality due to aortic dissection, cardiac evaluation is important every 5 years, even in patients with normal findings on initial cardiovascular examination.

Auditory testing

Infants diagnosed at birth should have a hearing assessment at the age of 1 year and before entering school. Re-evaluation is carried out every 5 years and more frequently in children with repeated otitis media. Adults should have a hearing evaluation at least once, with further testing later if hearing loss is suspected.

Bone age

Bone age is usually normal prior to adolescence but is delayed afterward because of the lack of estrogens. Bone age should be assessed before starting growth hormone or estrogen therapy. Growth hormone does not increase height if the epiphyses are fused, therefore, growth hormone is contraindicated if epiphyses are fused.

Bone mineral density (BMD) assessment: Osteoporosis is common and BMD is measured at diagnosis in adults and repeated every 3–5 years.

Prenatal diagnosis

On fetal ultrasonography, in the presence of a nuchal cystic hygroma, horseshoe kidney, left-sided cardiac anomalies, or nonimmune fetal hydrops, Turner syndrome should be suspected.[28] Lymphoedema at birth may also suggest Turner syndrome. There may also be increased nuchal translucency in the first trimester, nuchal fold in the second trimester. Turner syndrome may be prenatally diagnosed by amniocentesis or chorionic villous sampling or by non-invasive prenatal testing of maternal blood.[29]

Management

Turner syndrome is often diagnosed in early childhood because of the well-known phenotypic characteristics (short stature, webbed neck and low hairline) and the confirmation is done by karyotyping. **Management should be by a multidisciplinary team** involving gynaecologist, endocrinologist, cardiologist, nephrologist or urologist, psychologist and a genetics consultant.

General measures

Both short stature and ovarian failure are risk factors for osteoporosis, and care should be taken to ensure

adequate daily intake of calcium (1.0–1.5 g) and vitamin D (at least 400 IU). Patients should avoid obesity as they already are at risk of hypertension and insulin resistance. In the management of these patients, focus should be on improving the height, promoting breast development, correcting hypothyroidism and preventing osteoporosis.

Estrogen therapy

In order to achieve breast development, menstruation and adult height, as initial management, small doses of oestrogen with 2 μg ethinyl oestradiol alone is started around 10–12 years of age to promote maximum growth spurt before epiphyseal closure. The aim is to achieve bone age to 12–13. Bone age should be assessed periodically. Subsequently, oestrogen is given in a cyclical fashion with 3 weeks on and one week off for 2 years after which progestogens is added. Hormone therapy with 0.3/0.625 mg of conjugated equine oestrogen with medroxyprogesterone acetate given from days 1–10 to get regular withdrawal bleeding can also be given. Patients should be monitored periodically for possible side effects. Estrogen replacement therapy if started too early or if high doses are used can compromise adult height.

Thyroxine supplementation

These patients may also need replacement with thyroxine if there is associated hypothyroidism.

Growth hormone therapy

Growth hormone therapy is started in childhood to prevent short stature as an adult. The ideal age to initiate treatment is not known. Growth hormone can be initiated <10 years of age. **However, with longer duration of use with growth hormone taller height may be reached.** Even with growth hormone therapy, most individuals are shorter than average. Patients on growth hormone should be followed up every 3–6 months.[30]

Intellectual development

Females who have a 45, X karyotype usually have normal intelligence although there may be some learning disabilities, particularly with mathematical and spatial concepts, and some difficulty in social situations. Assessment of intelligence, learning ability, motor skills, and social maturity should be done periodically in children.

Psychological support

As adults they may need psychological support.

Pregnancy

Pregnancy can be achieved in these individuals with oocyte donation and embryo transfer. There are number of interesting developments in fertility preservation in women suffering from Turner syndrome which will be discussed later.

Panhypopituitarism

A number of conditions involving the hypothalamus and pituitary result in low levels of gonadotrophins and other hormones responsible for the growth of the individual. These individuals have normal karyotyping (46, XX), short statured with normal development of the gonads and genital tract. As the gonadotrophin levels are low, the ovaries are not stimulated, resulting in sexual infantilism. There may also be associated hypothyroidism, diabetes insipidus, and adreno-cortical insufficiency. In these individuals CT/MRI scan should be carried out to identify tumours, empty sella syndrome, craniopharyngioma, etc. When there is a definite cause such as tumours, it should be treated. In some of these cases, it is possible to achieve ovulation, menstruation and pregnancy with gonadotrophin therapy. These patients would require long-term hormone therapy for breast development and menstruation.

Congenital hydrocephalus/childhood hydrocephalus

Neonatal or childhood infection can result in hydrocephalus which can damage the hypothalamus. Similar hypothalamic damage can occur due to head injuries.

Empty sella syndrome

There is absence of sufficient amount of pituitary tissue, therefore, gonadotrophins are not secreted in response to GnRH from the hypothalamus resulting in primary amenorrhoea.

Tumours

a. Craniopharyngioma (Rathke's pouch tumour) present as nonpituitary suprasellar mass. They are diagnosed by CT/MRI. They cause hypopituitarism by directly destroying the pituitary or by interfering with the portal circulation. Besides amenorrhoea, they also present with visual and neurological symptoms. They are treated by surgery.
b. Tuberculosis and syphilitic gumma can also produce similar effects.

Other CNS dysfunctions

a. Laurence-Moon-Biedl syndrome: It is an autosomal recessive disease with hypothalamic dysfunction. Patients affected by this condition are short statured with severe obesity, mental retardation, sexual infantilism, polydactyly, coarctation of the aorta, retinitis pigmentosa and occasionally with diabetes insipidus and diabetes mellitus.
b. Prader-Willi syndrome: It is a hypothalamic defect with severe obesity, hypogonadism, and mental retardation. They have characteristic round facies, small broad hand and feet with infantile hypotonia.

Isolated GnRH deficiency and olfactogenital syndrome (Kallmann syndrome).

Here the hypothalamus lacks the ability to produce GnRH. The pituitary is normal and stimulation with GnRH leads to release of gonadotrophins. It is associated with anosmia. The aetiology of this condition is due to gene deletion which may occur sporadically or in families. The girl will be of normal height or tall statured as the effect of sex hormones on bone maturation and epiphyseal fusion is lacking or delayed. Adrenal androgens will induce moderate quantity of axillary and pubic hair. During investigations, space occupying lesions in the hypothalamus/pituitary region such as craniopharyngioma or chromophobe adenomas may be detected which may also cause isolated GnRH deficiency.

Management

Either the ovary can be stimulated with gonadotrophins or the pituitary with GnRH for stimulating follicular growth, ovulation, menstruation and pregnancy. Due to the long-standing deficiency of endogenous GnRH, GnRH stimulation may have to be given for a longer period of time before eliciting a gonadotrophin response. In these patients, hormone therapy would be required for long-term protection of bone.

Weight Loss/Anorexia Nervosa/Hyperprolactinaemia

Specific hypothalamic disorders are extremely rare causes of amenorrhea. However, there exist many common conditions which influence GnRH pulsatility and cause hypogonadotrophic amenorrhoea. Psychological stress, severe weight loss, chronic illness, acute severe illness and strenuous exercise suppress GnRH. Women suffering from advanced HIV disease often present with amenorrhea. These individuals have normal growth, and the fault lies in the pulsatile release of gonadotrophins because of the effect of low body mass and low fat on the secretion of GnRH. Hypothalamus has centres controlling over eating and under eating, therefore, obesity and wasting will affect menstruation. Most often hyperprolactinaemia results in secondary amenorrhoea. Rarely, prolactinomas may develop in pre-pubertal girls, resulting in primary amenorrhoea. Occasionally, sustained emotional stress can also lead to hyperprolactinaemia and resultant amenorrhoea. Under normal physiological conditions, prolactin remains suppressed under the influence of prolactin inhibitory factor (PIF-dopamine). Under extremes of stress this inhibitory influence is withdrawn and prolactin releasing factors such as serotonin and TRH are increased. The resultant hyperprolactinaemia can lead to primary amenorrhoea. Women involved in competitive sports activities have a three-fold higher risk of primary or secondary amenorrhoea than others, and the highest prevalence is among long-distance runners.[31]

Pure Gonadal Dysgenesis

There is failure of development of gonads and the chromosomal pattern is either 46, XX or 46, XY. Because of the absence of gonads, the individual develops into normal female phenotype. As there is no müllerian inhibiting factor, they have normal female internal and external genital organ development. Because of the deficiency of oestrogen, the serum gonadotrophin level is very high and there is sexual infantilism. They have normal or above normal height because of unopposed action of growth hormone in the absence of oestrogen resulting in delayed epiphyseal closure. There may be associated congenital deafness. In the XY pure gonadal dysgenesis, there is 25–30% risk of malignant tumours such as gonadoblastoma, therefore, gonadectomy should be performed soon after diagnosis.

Aetiology

- 46, XX PGD is transmitted by an autosomal recessive gene and parents are of consanguineous marriage. Several members of the siblings may be affected.
- 46, XY PGD (Swayer syndrome)

In this condition, there is loss of testicular determining factor in the Y chromosome or absence of its receptors due to SRY gene mutation. The condition is transmitted by an X-linked recessive or as an autosomal dominant gene expressed in females only. Can occur in siblings.

Management

They respond to hormone therapy and cyclical hormone therapy is given to achieve menstruation and breast development. Pregnancy can be achieved through oocyte donation.

Premature Ovarian Failure

Viral oophoritis, tuberculosis, autoimmune disease (as part of multiple autoimmune disease: Addison's disease, hypoparathyroidism, lymphocytic thyroiditis) and Galactosaemia (in born error of galactose metabolism due to the deficiency of galactose-1-phosphate uridyltransferase) can all lead to destruction of ovary and failure of ovarian function in childhood. Similarly, childhood malignancy, chemotherapy and radiotherapy can also destroy the ovarian tissue leading to hypogonadism and primary amenorrhoea. Treatment is similar to other conditions causing hypogonadism with cyclical hormone therapy.

Turner mosaic

In mosaicism two cell lines exist within the same individual. In Turner mosaic the karyotyping is either 45, XO/46, XX or 45, XO/46, XY. There could also be structural abnormalities or deletions. Deletion may be in the long arm of X (46, XX q) or short arm (46 XX p). Depending upon the proportion of each cell line, the manifestations will differ. The individual may be of normal height, structural abnormalities may be absent, the ovary may be dysgenetic

with hypogonadism. In the presence of some normal 46, XX cells, there is a possibility of ovarian differentiation and development of secondary sexual characteristics and a few individuals may conceive. Usually Turner mosaic presents as secondary amenorrhoea.

PRIMARY AMENORRHOEA WITH HETEROSEXUAL DEVELOPMENT

A number of conditions can present with primary amenorrhoea and heterosexual development:

1. Congenital adrenal hyperplasia
2. Adrenal tumours
3. PCOS
4. Androgenic ovarian tumours
5. Cryptorchid male intersex
6. True hermaphrodite
7. Virilising male intersex
8. Mixed gonadal dysgenesis

In individuals presenting with primary amenorrhoea and heterosexual development, karyotyping should be done as the initial evaluation.

- If the karyotyping is abnormal, conditions such as true hermaphroditism, mixed gonadal dysgenesis, virilising male intersex and cryptorchid male intersex should be considered.
- If the karyotyping is normal, then androgen and gonadotrophin levels are estimated to diagnose adrenal and ovarian causes for heterosexual development (Fig. 2.17).

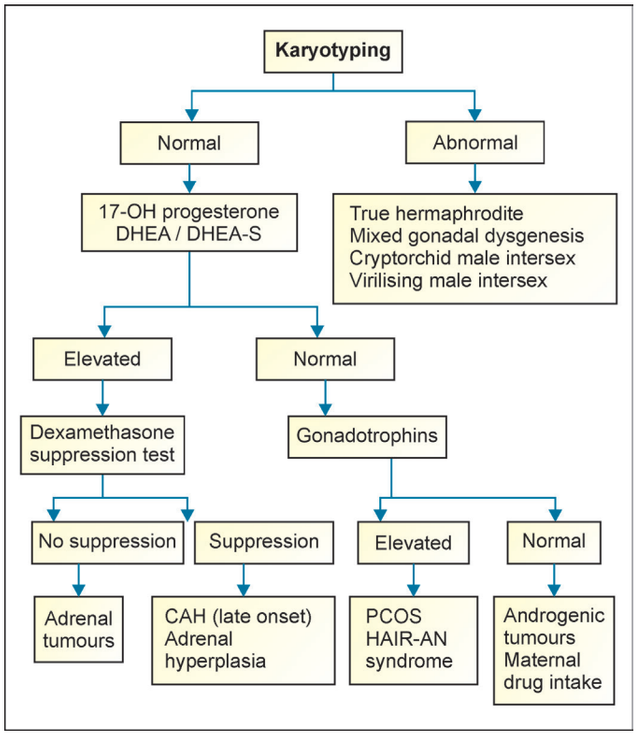

Fig. 2.17: Evaluation of primary amenorrhoea with heterosexual development

Congenital Adrenal Hyperplasia (CAH): Adrenogenital Syndrome/Pseudohermaphroditism

This is the most common condition, presenting at birth exhibiting heterosexual features with ambiguous external genitalia. It can also present initially at puberty with clitoral enlargement. This is called late onset or non-classic variety of CAH. This is an autosomal recessive disorder which leads to an enzyme deficiency in the synthesis of cortisol. The genes responsible for this disorder are located on the short arm of chromosome 6. 21-hydroxylase deficiency is the most common deficiency, but 11-hydroxylase deficiency can also occur.

- In the steroid biosynthesis pathway, for the conversion of progesterone and 17-OH progesterone to 11-deoxycorticosterone and 11-deoxycortisol, 21β-hydroxylase enzyme is required. In the absence of 21-hydroxylase enzyme, there is failure to produce cortisol in normal quantities. This leads to compensatory increase in ACTH production which leads to increase in 17-OH progesterone, androstenedione and DHEA. These androgens are converted to testosterone in the peripheral tissue leading to hirsutism and virilisation. As there is failure of conversion of progesterone to 11-deoxycorticosterone, there is also aldosterone deficiency. These individuals are short statured due to early epiphyseal closure because of the high androgen levels. Usually presents as ambiguous external genitalia in the newborn period. There is variable degree of masculinisation with fused labia. There is non-canalisation of the lower part of uro-genital sinus. Internal genital organs are normal. There is also skin pigmentation.
- In the non-classical variety, there is virilizing puberty, obesity, acne, hirsutism, primary or secondary amenorrhoea. Elevated 17-OH progesterone levels are diagnostic of 21-hydroxylase deficiency.
- Some of the newborns may have salt loosing type of CAH, they present with hypotension, hyponatremia which usually manifests at 2nd year of life. They also manifest hypoglycaemia, and electrolyte imbalance and treated with glucose, isotonic saline and steroids. They need life-long treatment.

Treatment

- ACTH levels should be continuously suppressed with cortisone 50 mg/day. Plastic reconstruction of external genital organs and clitoridectomy should be undertaken before the child recognizes the variation in external genitalia.
- In cases with 11-hydroxylase deficiency, there is accumulation of 11-deoxycortisol and deoxycorticosterone which are weak mineralocorticoids. The 11-hydroxylase

deficiency is due to mutation of gene located on the long arm of chromosome 8. Due to increased androgen levels, there is ambiguity of external genitalia. Due to increased mineralocorticoids, there is hypertension and hypokalaemia. These patients are treated by giving glucocorticoids.

Prenatal diagnosis

When there is a history of affected sibling, prenatal diagnosis can be undertaken by measuring 17-OH progesterone levels in the amniotic fluid. If raised it can be treated with dexamethasone to suppress the fetal adrenals. DNA probes are also available to diagnose CAH which can be used after chorion villous sampling. If the above facilities are not available and the sex of the foetus is not known, dexamethasone is started as soon as pregnancy is confirmed and continued until delivery.

Cushing's Syndrome

There is hyperfunction of the entire adrenal cortex including cortisone secreting zona fasciculata. It may be due to the primary pituitary dysfunction or due to an adrenal tumour. Pituitary dysfunction may be due to pituitary adenoma or hyperplasia or ectopic ACTH secretion from bronchogenic carcinoma. These patients present with progressive central obesity (moon facies), striae, fragile skin, ecchymosis, acne, mild hirsutism, clitoromegaly, hypertension, glucose intolerance, hyperinsulinaemia, osteoporosis, primary or secondary amenorrhoea. These clinical features are seen in adrenal tumours also. Imaging modalities with CT/MRI scan will help in locating the lesion. Overnight dexamethasone suppression test will differentiate adrenal tumours from adrenal hyperplasia secondary to pituitary dysfunction. 24 hours urinary free cortisol and serum AM and PM cortisol levels are increased.

Adrenal Tumours

Adrenal tumours could be adenomas and carcinomas. They secrete DHEA-S and androstenedione which are converted to testosterone peripherally. Elevated DHEA-S levels and markedly elevated testosterone levels indicate adrenal tumours. The levels do not reduce with dexamethasone suppression test. The tumours are removed surgically.

Androgen Producing Tumours of the Ovary

Sex cord tumours such as Sertoli-Leydig cell tumours, hilus cell tumours, steroid cell tumours, lipoid cell tumours and arrhenoblastomas can present as primary/secondary amenorrhoea. Patients present with progressive and rapidly increasing hirsutism, virilisation and abdomino-pelvic mass. Testosterone level is markedly elevated.

Cryptorchid Male Intersex

5α-reductase insufficiency (Fig. 2.18)

This is an incomplete variety of testicular feminisation. The individual has normal karyotype with 46, XY. The testes develop normally, therefore, there is normal secretion of anti-müllerian hormone and testosterone. Under the influence of anti-müllerian hormone the müllerian structures regress and under the effect of testosterone, the internal male genital organs develop. However, due to the deficiency of 5α-reductase enzyme, testosterone is not converted to dihydrotestosterone, therefore, the external genitalia is either feminine or ambiguous. At puberty, testosterone causes rapid virilisation of the external genitalia, in an individual who was reared as a female child, because of the feminine external genitalia. There will be growth of penis and descent of testes into the scrotum. It is difficult to make decisions in the management of these cases. These cases can be reared as females following gonadectomy and reconstruction of genitalia. If the assigned sex is female, testes should be removed before puberty to prevent masculinising puberty. Excision of the enlarged phallus should be undertaken before the child notices the change. Exogenous steroids would be required to induce feminization. Artificial vagina should be constructed for sexual function. They can also be reared as males because of the normal testosterone, normal male internal genital organs and virilising puberty. However, the external genital organs will be ambiguous.

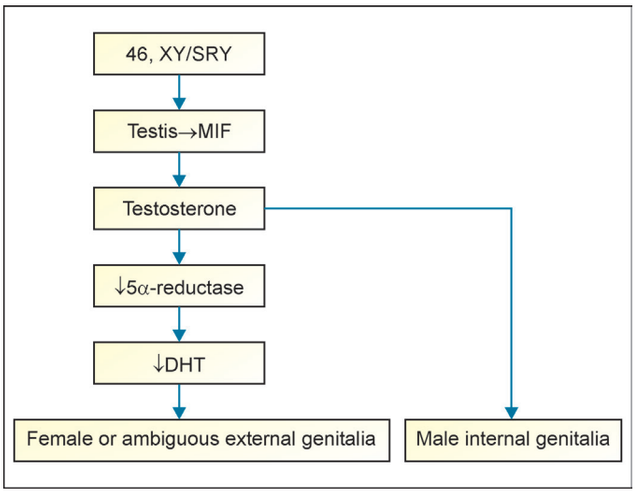

Fig. 2.18: Heterosexual development in 5α-reductase deficiency

Virilising Male Intersex

These individuals have male karyotyping 46, XY and normal development of the testes. However, the testes lack the anti-müllerian factor, therefore, the individual develops uterus, fallopian tubes and the vagina. There is also partial cloacal resistance to normal intrauterine androgen production. Therefore, at birth there is ambiguous external genitalia with enlarged clitoris with variable degree of labioscrotal fusion and a urogenital sinus. Bilateral testes are present in the ovarian fossa,

inguinal canal or labial fold. If untreated they have virilizing puberty (Fig. 2.19).

Mixed Gonadal Dysgenesis

The karyotype is XO/XY, the gonad is unilateral testes with contralateral streak or gonad or gonad plus tumour such as dysgerminoma, seminoma and gonadoblastoma. There is ambiguous external genitalia at birth. Internal sex is feminine, external sex is equivocal and sex of rearing is usually female. Treatment is by gonadectomy and clitoridectomy.

True Hermaphrodite

Both testicular and ovarian tissue containing primordial follicles and seminiferous tubules are present in the same individual. They have either bilateral ova testis or ovary on one side and testes on the other side. Karyotyping can be 46, XX/46, XY/47, XXY, 46, XX/48, XXYY. They have normal uterus and breast development and even menstruation can occur if ovarian element functions. Most commonly they present with menstruation in association with male external genitalia at puberty.

RECENT DEVELOPMENTS IN THE MANAGEMENT OF PRIMARY AMENORRHOEA

There have been interesting developments in areas of fertility preservation in women presenting with primary amenorrhoea.

Uterine Transplantation

Since the first birth of a child after uterus transplantation in 2014, there has been interest in treating müllerian agenesis with uterine transplantation. Though the procedure is not widely available and there is limited data on the success, it may provide hope for the future.

Fertility Preservation in Turner Syndrome

Oocyte cryopreservation: In girls with classic Turner syndrome with 45, XO with no mosaicism, the ovarian primordial follicle reserve is depleted well before puberty. In Turner syndrome with mosaicism, depending on the degree of mosaicism, ovarian reserve may persist for a variable period of time during childhood and following menarche. However, this reserve would be low and can get depleted rapidly. Therefore, to preserve these oocytes, ovarian stimulation followed by oocyte retrieval and cryopreservation have been reported. It has been recommended that all girls with Turner syndrome, if they attain menarche should be evaluated for ovarian reserve. In these individuals, both patients and families should be counseled regarding oocyte cryopreservation as a viable fertility preservation option. For those who have already lost their ovarian reserve, donor oocytes, donated embryos and adoption are strategies to bear children.[32]

Fig. 2.19: Ambiguous external genitalia

Ovarian Tissue Cryopreservation

For the classic non-mosaic cases, where ovarian reserve can be depleted within the first few years of life, the referral should occur as soon as a diagnosis is made, even in infancy. In these pre-pubertal girls, ovarian tissue cryopreservation (OTCP) has been recommended.[33]

Ovarian Transplantation

Ovarian transplantations have also been attempted on patients diagnosed to have Turner's syndrome using ovarian tissue donated by mother/sister.[34]

Ethical Issues

In children born with ambiguous external genitalia ethical issues will arise as to whether to perform reconstructive surgery in infancy or to delay it until children are old enough to decide for themselves.

KEY POINTS

- Basic factors involved in the initiation and continuation of normal menstruation are normal female chromosomal pattern 46, XX, normal development of the ovary, co-ordinated HPO axis functioning, anatomical patency of the genital tract, active support of the adrenal and thyroid glands and responsive endometrium.
- Primary amenorrhea is defined as the absence of menstruation in a girl by 16 years of age.
- Indications for early evaluation include failure of development of breast and/or secondary sexual characteristics by 13–14 years of age, when there is evidence of virilisation, when the individual is short-statured and when the girl presents with cyclical abdominal pain with retention of urine and/or difficulty in defaecation.
- History and detailed examination would indicate the investigations that would be required to diagnose primary amenorrhoea.
- MRI is considered the gold standard for diagnosis of müllerian abnormalities.

(Contd.)

(Contd.)

KEY POINTS

- Though the causes of primary amenorrhoea are classified based on the site of pathology, for practical purposes and for clinical evaluation, cases are divided into three groups based on the secondary sexual characteristics.
- In those diagnosed with sexual infantilism, cyclic estrogen and progestogen therapy should be given for the initiation, maturation, and maintenance of secondary sexual characteristics and for the maintenance of bone health.
- In those with 'Y' chromosomes, gonadectomy should be done to prevent malignancy.
- In those with absent vagina, neo vagina should be created for sexual function.
- The possibility of fertility preservation and pregnancy should be explored in all cases of primary amenorrhoea.
- In those presenting with ambiguous external genitalia, sex of rearing should be decided in childhood after necessary investigations.

Case Scenario 1

1. A 17-year-old adolescent girl was brought to the OPD by her mother as she had not yet attained menarche and there were no other complaints. She has one younger sister who has attained menarche at the age of 13. There was no significant childhood/medical history. On examination, she was 160 cm tall, weighed 60 kg and there was no thyroid enlargement. The breast, axillary and pubic hair development were normal with Tanner stage IV. The external genitalia were feminine.

   a. What are the possible causes of primary amenorrhoea in this girl and what is the initial investigation you will order?

      In any individual presenting with normal secondary sexual characteristics, it indicates that the HPO axis is functional and the ovaries are secreting oestrogen. The possible causes in this scenario would be müllerian agenesis, end-organ failure as in genital tuberculosis, constitutional delay, pre-pubertal PCOS and hypothyroidism. Though testicular feminization presents with normal breast development. Pubic and axillary hair will be absent or scanty due to the lack of androgen receptors. In these cases, the initial investigation should be USG of the pelvis to evaluate the genital tract.

   b. USG of the abdomen and pelvis showed absent uterus, and one kidney was in the pelvic position. Local examination showed the vagina to be blind. What are the conditions should be considered in an individual with normal breast development and absent uterus with blind vagina? What further investigations should be ordered?

      The most common causes of primary amenorrhoea in individuals with normal breast development with absent uterus and vagina are müllerian agenesis and testicular feminization syndrome. Though the presence of Tanner IV axillary and pubic hair development rules out testicular feminization, the diagnosis of müllerian agenesis is confirmed by karyotyping which is 46, XX (in testicular feminization it is 46, XY) and normal levels of testosterone (increased in testicular feminization). Urinary tract anomalies are seen in nearly 30–40% of cases.

Case Scenario 2

2. A 16-year-old girl was referred by the GP for not having attained menarche. On examination, her height was 140 cm and she weighed 49 kg. She had webbing of the neck and there was polydactyly of both hands. Her breast and pubic hair development were Tanner stage I. Abdominal examination was normal and external genitalia was infantile.

   a. What is the possible diagnosis in this case and what are the causes of sexual infantilism?

      The possible diagnosis in this case is Turner syndrome as the individual is short statured with sexual infantilism and presenting with somatic features. Panhypopituitarism due to trauma, hydrocephalus, empty sella syndrome, also present with short stature and sexual infantilism, but there are no somatic abnormalities. Diagnosis is confirmed by karyotyping and measuring the gonadotrophin levels. The karyotyping is 45, XO in Turner syndrome and is 46, XX in panhypopituitarism. The gonadotrophin levels are increased in Turner syndrome, whereas it is low in panhypopituitarism. The other causes of sexual infantilism are isolated GnRH deficiency, ovarian failure due to autoimmune diseases, tuberculosis and mumps which can destroy the ovaries. There are also other causes such as pure gonadal agenesis and Turner mosaics.

   b. What are the goals of management in treating Turner syndrome?

      The management of these patients should focus on improving the height, promoting breast development, correcting hypothyroidism and preventing osteoporosis. In order to achieve breast development, menstruation and adult height, as initial management, small doses of oestrogen with 2 mg ethinyl oestradiol alone is started around 10–12 years of age to promote maximum growth spurt before epiphyseal closure. Subsequently, oestrogen is given in a cyclical fashion with 3 weeks on and one week off for 2 years after which progestogen is added. Growth hormone therapy is started in childhood to prevent short stature as an adult. Growth hormone can be initiated <10 years of age.

REFERENCES

1. Stévant I, Nef S. Genetic Control of Gonadal Sex Determination and Development. *Trends Genet* 2019;35:346–358.
2. Abreu AP, Kaiser UB. Pubertal development and regulation. *Lancet Diabetes Endocrinol* 2016; 4: 254–264.
3. Gasner A, Rehman A. Primary Amenorrhea. [Updated 2023 Mar 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554469/Accessed on 12/10/23
4. Leone T, Brown LJ. Timing and determinants of age at menarche in low-income and middle-income countries. *BMJ Glob Health* 2020; 5: e003689.
5. Garnier D, Simondon KB, Bénéfice E. Longitudinal estimates of puberty timing in Senegalese adolescent girls. *Am J Hum Biol* 2005;17:718–730.
6. Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM. Secondary sexual characteristics and menses in young girls seen in office practice: A study from the Pediatric Research in Office Settings network. *Pediatrics* 1997;99:505–512.
7. Kota AS, Ejaz S. Precocious Puberty. [Updated 2023 Jul 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544313/
8. Folch M, Pigem I, Konje JC. müllerian agenesis: etiology, diagnosis, and management. *Obstet Gynecol Surv* 2000;55: 644–649.
9. Quint EH, McCarthy JD, Smith YR. Vaginal surgery for congenital anomalies. *Clin Obstet Gynecol* 2010;53:115–124.
10. Connell M, Owen C, Segars J. Genetic Syndromes and Genes Involved in the Development of the Female Reproductive Tract: A Possible Role for Gene Therapy. *J Genet Syndr Gene Ther* 2013;4:127.
11. Parul Shah. A study of primary amenorrhoea. *JOGI* 1992;42: 275–277.
12. Thangappah Radha Bai Prabhu, Chandralekha. Cytogenetic, Morphological and Metabolic Abnormalities in Primary Amenorrhoea. *JOGI 2004;*54:167–169 (2004).
13. Herlin MK, Petersen MB, Brännström M. Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome: a comprehensive update. *Orphanet J Rare Dis* 2020;15:214.
14. Rousset P, Raudrant D, Peyron N, Buy JN, Valette PJ, Hoeffel C. Ultrasonography and MRI features of the Mayer-Rokitansky-Küster-Hauser syndrome. *Clin Radiol* 2013;68: 945–952.
15. Sultan C, Biason-Lauber A, Philibert P. Mayer-Rokitansky-Kuster-Hauser syndrome: Recent clinical and genetic findings. *Gynecol Endocrinol* 2009; 25: 8–11.
16. The Obstetrician and Gynaecologist Saudabi Valappil, Uma Chetan, Nick wood, Anne Garden. Mayer-Rokitansky-Kuster-Hauser Syndrome, diagnosis and management 2012;14:93–98.
17. Herlin M, Bjørn AM, Rasmussen M, Trolle B, Petersen MB. Prevalence and patient characteristics of Mayer-Rokitansky-Küster-Hauser syndrome: a nationwide registry-based study. *Hum Reprod* 2016;31:2384–2390.
18. Mahendra N Parikh. Congenital absence of Vagina: MRKH Syndrome. *JOGI* 2000; 50:128–138.
19. Friedler S, Grin L, Liberti G, Saar-Ryss B, Rabinson Y, Meltzer S. The reproductive potential of patients with Mayer-Rokitansky-Küster-Hauser syndrome using gestational surrogacy: a systematic review. *Reprod Biomed Online*. 2016; Jan. 32:54–61.
20. Flyckt R, Davis A, Farrell R, Zimberg S, Tzakis A, Falcone T. Uterine Transplantation: Surgical Innovation in the Treatment of Uterine Factor Infertility. *J Obstet Gynaecol Can* 2018;40: 86–93.
21. American College of Obstetricians and Gynecologists. Müllerian agenesis: diagnosis, management, and treatment. *Obs Gynecol* 2018;131:35–42.
22. Gîngu C, Dick A, P tr coiu S, Domni or L, Mihai M, Hârza M, Sinescu I. Testicular feminization: complete androgen insensitivity syndrome. Discussions based on a case report. *Rom J Morphol Embryol* 2014;55:177–181.
23. Singh S, Ilyayeva S. Androgen Insensitivity Syndrome. 2023 Feb 28. In: StatPearls. Treasure Island (FL): *Stat Pearls Publishing*; 2023 Jan–. PMID: 31194363.
24. Shankar Kikkeri N, Nagalli S. Turner Syndrome. [Updated 2023 Aug 8]. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554621/Accessed on 13/10/23
25. Donaldson MD, Gault EJ, Tan KW, Dunger DB. Optimising management in Turner syndrome: from infancy to adult transfer. *Arch Dis Child* 2006;91:513–520.
26. Zeng Y, Li L, Li Q, Hu J, Zhang N, Wu L, Yan Z, Qu R, Dong J, Liu R, Choy KW, Wang L, Sang Q, Guan Y, Chen B. Genetic screening in patients with ovarian dysfunction. *Clin Genet* 2023;103:352–357.
27. Bianco B, Lipay MV, Melaragno MI, Guedes AD, Verreschi IT. Detection of hidden Y mosaicism in Turner's syndrome: importance in the prevention of gonadoblastoma. *J Pediatr Endocrinol Metab* 2006;19:1113–1117.
28. Sutton EJ, McInerney-Leo A, Bondy CA, Gollust SE, King D, Biesecker B. Turner syndrome: four challenges across the lifespan. *Am J Med Genet A* 2005;139:57–66.
29. Alpman A, Cogulu O, Akgul M, Arikan EA, Durmaz B, Karaca E, Sa ol S, Ozkinay C, Ozkinay F. Prenatally diagnosed Turner syndrome and cystic hygroma: incidence and reasons for referrals. *Fetal Diagn Ther* 2009;25:58–61.
30. Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol* 2015;45:249–266.
31. Bolar K, Hoffman AR, Maneatis T, Lippe B. Long-term safety of recombinant human growth hormone in turner syndrome. *J Clin Endocrinol Metab* 2008;93:344–351.
32. Warren MP, Goodman LR. Exercise-induced endocrine pathologies. *J Endocrinol Invest* 2003;26:873–878.
33. Oktay K, Bedoschi G, Berkowitz K, Bronson R, Kashani B, McGovern P, Pal L, Quinn G, Rubin K. Fertility Preservation in Women with Turner Syndrome: A Comprehensive Review and Practical Guidelines. *J Pediatr Adolesc Gynecol* 2016;29: 409–416.
34. Jeve YB, Gelbaya T, Fatum M. Time to consider ovarian tissue cryopreservation for girls with Turner's syndrome: An opinion paper. *Hum Reprod Open* 2019; 20: hoz016.
35. Ramesh V, Pal C. Organ Donation and Transplantation in India in 2019. *Exp Clin Transplant*. 2021;19:1313–1321.