Monograph

An Update on the Causes of Primary and Secondary Amenorrhea along with Aetiopathogenesis and Therapeutic Management

Kulvinder Kochar Kaur¹*, Gautam Allahbadia² and Mandeep Singh³

¹Dr. Kulvinder Kaur Centre for Human Reproduction, India
²Rotunda-A Centre for Human Reproduction, India
³Swami Satyanand Hospital, India

*Corresponding Author: Kulvinder Kochar Kaur, Dr. Kulvinder Kaur Centre for Human Reproduction, 721, G.T.B. Nagar, Jalandhar-144001, Punjab, India; Tel: 91-181-9501358180/91-181-4613422; Fax: 91-181-4613422; Email: kulvinder.dr@gmail.com

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Abstract

Amenorrhea is defined as the absence of periods by age 14 without development of secondary sex characteristics and age 16 irrespective of development of secondary sex characteristics. Secondary Amenorrhea is the absence of menses for 3 consecutive cycles. This monograph comprehensively summarizes the various causes of both primary and secondary amenorrhea, be it at uterine level, ovarian, pituitary or hypothalamic level. Step by step evaluation as well as detailed causes at each level is discussed in details from aetiopathogenesis, endocrinological and management point of view.

Keywords

Primary Amenorrhea; Secondary Amenorrhea; Uterine end organ insensitivity, Ashermann Syndrome, Turners Syndrome; P.O.F; Pituitary Tumours with Hyperpeolactinemia; Hypothalamic Amenorrhea; Anorexia Nervosa; Kallmanns Syndrome; NIHH
Introduction

Amenorrhea can be divided into primary and secondary

Definition

Primary Amenorrhea (PA) is defined if a patient does not get periods by the age of 14 in the absence of secondary sex characters or ii) if there are no periods by age 16 irrespective of secondary sex characters having got developed. iii) Secondary Amenorrhea (SA) is the diagnosis in a woman who had been menstruating earlier; if she has absence of periods for a length of time equivalent to a total of at least 3 of the previous cycle intervals or 6 months of amenorrhea, a diagnosis of amenorrhea is made and she needs to be evaluated.

Normal Physiology Behind Menstrual Function

For menstrual function and visible menstrual discharge an intact outflow tract is required which connects the internal genital source of outflow with outside. This requires patency along with continuity of the vaginal orifice, the vaginal canal and the endo cervix with the uterine cavity. For menstrual flow to occur there has to be an existence of, along with development of the endometrial lining in the uterine cavity. This is dependent upon the appropriate quantity along with sequence of the two steroid hormones namely estrogen and progesterone. The origin occurs in the ovary, specifically in the developing follicle, underlying ovulation and corpus luteum function. The maturation of the follicular apparatus is guided by the stimuli provided by the stimuli provided along with the sequence along with the magnitude of gonadotropins, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH) which originates in the anterior pituitary. Their secretion in turn is dependent on the specific peptide releasing hormone gonadotropin releasing hormone (Gn RH) produced by the basal hypothalamus which is blood borne via the portal vessels of the stalk to the receptive cells within the anterior pituitary. This entire system is regulated by a complex mechanism which integrates biophysical and biochemical information composed of interactive levels of hormone signals, autocrine, paracrine factors and target cell reactions.

Normally around 6-8 weeks of gestation evidence of ovarian differentiation gets reflected in the rapid mitotic multiplication of germ cells which reach around 6-7 million oogonia by 16-20 weeks [1,2]. This represents the maximal oogonial content of the gonad. From this time in life the germ cell content will keep decreasing till around 50 years when the oocyte content is finally depleted. Chromosomal anomalies like Turners syndrome can accelerate germ cell loss where there is normal migration as well as mitosis but there is no meiosis of the oogonia and rapid loss of oocytes from gonads occurs leaving the gonads without follicles, which appear as a fibrotic streak.
In patients with amenorrhea one needs to evaluate on the basis of various compartments with

Compartment I) Disorders of the outflow tract/uterine target organ
Compartment II) Disorders of the ovary
Compartment III) Disorders of the anterior Pituitary
Compartment IV) Disorders of the CNS (hypothalamic factors)

**Investigations of a Patient of Amenorrhea**

**Step 1**

Detailed history and physical examination including evidence of psychogenic dysfunction or emotional stress, family history of apparent genetic anomalies with a focus on nutritional status, abnormal growth and development, the presence of a normal reproductive tract and evidence of a CNS disease.

This is followed by step by step investigations, following exclusion of pregnancy, starting with serum TSH, Prolactin levels and a progesterone challenge test. If galactorrhea accompanies amenorrhea, then sellar imaging is included.

The duration of hypothyroidism is important with respect to mechanism of galactorrhea; longer the duration, greater is the incidence of galactorrhea and greater the prolactin levels [3]. This is believed to be due to decreasing hypothalamic dopamine content with ongoing hypothyroidism. This leads to an unopposed TRH stimulatory effect on the pituitary cells that secrete prolactin. Usually prolactin levels associated with hypothyroidism are <100ng/ml. Once there is a constant stimulation of hypothalamic releasing hormones, it leads to hypertrophy or hyperplasia of the pituitary. Thus the imaging picture of a tumor be it distortion, expansion or erosion of the sella turcica can be seen. Hence in primary hypothyroidism as well as in patients with elevated GnRH and gonadotropin secretion due to P.O.F., imaging studies in the form of computed tomography (CT scan or magnetic resonance imaging (MRI) is indicated [4,5]. Proper treatment is followed by rapid normalization of the initial picture. Patients with primary hypothyroidism and hyperprolactinemia can either present with primary or with secondary amenorrhea [6].

Next a progesterone challenge test is done to see the endogenous estrogen along with the competence of the outflow tract. A progestational agent course totally devoid of estrogenic activity is given. It can be given either parenterally as progesterone (P) in oil in a dose of 200mg, or orally as micronized P, 300mg/d, or as orally active medroxy progesterone acetate, in a dose of 10mg/d for 5days. Oral administration avoids the unpleasant injection (only necessary if compliance is a problem). Within 2-7days of stoppage of P the patient will bleed or not bleed. If bleeding occurs a diagnosis of anovulation is reliably established, along with the presence of a functional
outflow tract and a uterus lined by reactive endometrium. Once the presence of estrogen is demonstrated, minimal function of ovary, pituitary and CNS gets established.

The amount of bleeding is important as well. Very few spots following P suggest only marginal levels of endogenous estrogen. These patients need a close follow up, as the marginally positive response can progress to a clearly negative response and patient gets placed in a new category. Bleeding in any amount beyond a few drops is considered a positive withdrawl response. There are two situations which can be associated with a negative withdrawl response despite adequate endogenous estrogen. Both have an adequately decidualized endometrium and hence will not shed with the withdrawal of the exogenous P. The first is secondary to high androgen levels. In the second the endometrium is decidualized either due to high androgen or progesterone associated with a specific adrenal enzyme deficiency. This clinical response is not observed infrequently in patients with significant hyperandrogenemia, which is associated with anovulation and polycystic ovaries. Despite this occurring in young women a latent period from atypia to cancer is very small even in younger women precautions need to be taken, although for breast cancer it is a long period and some studies question the association of anovulation with development of breast cancer. When young if women are anovulatory, they may have a risk of breast cancer when they are postmenopausal [7].

Minimal treatment for anovulatory woman is a monthly administration of a progestational agent with an easy regimen giving medroxy progesterone acetate for first 2 weeks of the month. Time period of over 10 days P is shown to be protective against the unopposed effect of E2. If contraception is required then low dose oral contraceptive pill is appropriate in the usual cyclic fashion.

If at any time a patient fails to have withdrawl bleed in absence of pregnancy it means patient has shifted to the next category of negative withdrawl bleed and further followup is required. Occasionally progestational challenge will trigger an ovulation—the clue will be withdrawal bleed after 14 days of the progestational challenge. In the absence of galactorrhea and a normal serum prolactin, nothing further has to be done, except treating the anovulatory status. Rarely ectopic prolactin can be produced from pituitary tissue in base of pharynx, bronchogenic carcinoma, renal cell carcinoma, gonadoblastoma which can lead to hyperprolactinemia and amenorrhea due to a prolactinoma in the wall of dermoid cyst [8-12].

Step 2

If there is a negative P withdrawl bleed that means the target outflow tract is inoperative or that the preliminary estrogen (E2) proliferation of the endometrium has not occurred. To clarify this orally active E2 is administered in the form of conjugated estrogen 1.25mg/estradiol valerate 2mg, for 21 days. Terminally oral medroxy P acetate is
given as 10mg od for 5 days, which is essential to achieve withdrawal bleed. In case of absence of withdrawal bleed a precautionary 2nd dose is given. By this the compartment 1 gets challenged by exogenous E2. With this the amenorrheic patient will bleed or not. With no withdrawal bleed a defect in compartment 1 can be made with confidence. If bleeding occurs that means the compartment 1 is normal. Practically in patients with normal external and internal genitalia on pelvic examination and absence of history of infection or trauma (e.g. curettage) an abnormality of the outflow tract is not likely. Abnormalities of the compartment 1 are not commonly encountered and in absence of reason to suspect one can omit step 2.

**Step 3**

If an amenorrheic patient is unable to produce adequate E2, the physiological mechanism for steroid elaboration needs to be tested. To produce E2 ovaries containing a normal follicular apparatus, besides sufficient gonadotropins to stimulate this apparatus is needed. Step 3 is to test where there is malfunction in these 2 components. In this gonadotropin assay is required. Since in step 2 exogenous E2 is given step 3 should be delayed 2 weeks from step 2. A midcycle LH surge is 3 times normal hence if no withdrawal bleed occurs 2 weeks following that then that LH level is abnormal. The gonadotrophin levels will be abnormally high (FSH>20iu/l, LH>40iu/l, abnormally low (Both FSH and LH<5iu/l), or within normal range (5-20iu/l), in those not getting a progesterone withdrawal bleed.

**Compartment I:- Disorders of Out-Flow Tract**

**Ashermanns Syndrome (AS)**

This usually follows excessive postpartum curettage which leads to extensive scarification and leads to secondary amenorrhea [13]. Hysterogram shows a typical pattern of multiple synchiae. Hysteroscopic diagnosis is more accurate as it detects even minimal adhesions, which are not seen on ahysterogram. The adhesions may partially or completely obliterate the endometrial cavity, internal cervical os, cervical canal or combination of these areas. Despite the stenosis or atresia of cervical canal, somehow haematometra does not occur. The endometrium in response to buildup of pressure becomes refractory and does not respond to a buildup of pressure, becomes refractory and this may get cured by a simple dilatation. AS can also occur following a caesarian section, a myomectomy or metroplasty. Very severe adhesions have been seen in a postpartum curettage or postpartum hypogonadism as in Sheehan’s syndrome. Following uterine embolization for fibroids the ischaemic response following the procedure causes endometrial damage and AS and thus secondary infertility [14]. Besides amenorrhea patients with AS may present with dysmenorrhea, miscarriages or hypomenorrhea. Their may be even normal menses. Patients may have minimal adhesions and infertility associated with
this cannot be easily explained. Those with repeated miscarriages, infertility or pregnancy wastage need investigation with either a hystero gram or preferably a hysteroscopy which can simultaneously treat them.

This impairment of uterine cavity can be caused by infections like tuberculosis which is more common in India and less in the west; To diagnose it culture, polymerase chain reaction (PCR) of the menstrual blood or tissue obtained from biopsy is used. Rarely uterine schistomiasis causes end organ failure, with eggs of the parasite found in urine, faeces, rectal scrapings, menstrual blood or endometrium.

In the past AS was treated using dilatation and curettage to break the intrauterine synechiae followed by a Cu IUCD inserted to reprevent development of new synechiae, but recently a paediatric Foley’s catheter is used with the balloon dilated with 3ml of fluid and catheter is removed after 7 days. Patient is put on a broad spectrum antibiotic preoperatively, which is continued for 10 days. To prevent cramping a prostaglandin synthetase inhibitor is used. Treatment with high doses of stimulatory E2 for 2 months (use of conjugated E2 2.5mg for first 3 weeks and medroxy progesterone acetate 10mg od added in the 3rd of 4th week). If initial attempts fail to establish menstrual flow, repeated attempts are worthwhile. Persistent treatment with repeated procedures may regain reproductive potential. 70-80% patients achieve a successful pregnancy approximately. In these patients pregnancy is frequently complicated by Preterm labour, Placenta accreta, placenta praevia, postpartum haemorrhage.

Mullerian Anomalies

In patients presenting with primary amenorrhoea mullerian tube segmental disruption should be ruled out. Hence, imperforate hymen, obliteration of vaginal orifices or lapses in continuity of the vaginal canal should be ruled out by direct observation. Their may be complete absence of the uterus or cervix. Much less common is a situation where uterus is present, but the cavity is absent or in the presence of a cavity the endometrium maybe lacking congenitally. With the latter exception such patients clinically not only present with amenorrhoea but in addition a haematocolpos, haematometra, or haemoperitoneum. In all such cases one must try to incise and drain from below at the point of the mullerian tube closure. Even in complicated cases continuity can be established surgically. Prior to draining knowing the full anatomy with the help of an MRI can be used to delineate the abnormality accurately [15,16].

Mullerian Agenesis-Mayer Rokitansky–Kuster–Hauser Syndrome is the diagnosis for an individual presenting with lack of mullerian development in a patient presenting with primary amenorrhoea and no apparent vagina [17]. This is a more common cause of primary amenorrhoea, commoner than androgen insensitivity syndrome (1 in 5000 newborn girls) [18]. These patients have an absence or a hypoplasia of the vagina, with usually absence of uterus and fallopian tubes. Rarely, the uterus
may be normal but lacking a passage to the introitus, or there maybe just rudimentary, bicornuate cords present. Incase of partial, endometrial cavity, cyclical abdominal pain may be a complaint. It is important to get a female karyotype because of similarities of some male pseudohypogonadism. Since ovaries are not a mullerian structure they are usually normal with normal BBT.

Causes of mullerian agenesis (MA) is unknown, but can be due to a mutation in the gene of the anti mullerian hormone or the AMH receptor. The underlying mechanism would be exposure to AMH acivity. No activating mutation is reported, in contrast to inactivating mutations which cause persistence of mullerian structures [19]. Amutation has been identified in galactose-1-phosphate uridyl transferase in daughters with mullerian agenesis as well as their mothers [20]. This is different from patients of classic galactosemia, though it is postulated that increased intrauterine exposure to galactose because of this error in galactose metabolism can be the biologic mechanism for mullerian agenesis, in this group of patients. High galactose feeding of pregnant mice delays vaginal opening in female offsprings. In such group of MA, oocyte depletion leading to P.O.F may be more common.

Approximately one third have urinary tract anomalies, of which renal abnormalities include ectopic kidney, renal agenesis, horse shoe kidney and abnormal collecting ducts. 12% have skeletal abnormalities, mostly involving the spine, though absence of digits and syndactyly i.e. webbing or fusion of fingers or toes can occur. When the anatomic picture regarding the presence or absence of uterus is not clear on ultrasonography (USG), then an MRI can be used [21,22] to confirm the anatomic findings. Laparoscopy is usually not required. MRI is more accurate than USG and less expensive as well as invasive than laparoscopy; however one study found disparity between MRI and laparoscopic findings half the time [23].

Because of the difficulties along with complications in surgical series, an alternative method to the surgical construction is favoured by some authorities. Use of progressive dilatation as was initially described by Frank [24] and later by Wabrek et al [25]. To start with one begins in the posterior direction and then after 2 weeks changing direction upwards towards the usual line of vaginal axis, pressure is applied with dilators available commercially for 20'/day to the point of modest discomfort. Gradually utilizing larger dilator a functional vagina can be created in several months [26,27]. Plastic syringe covers can be used instead of the expensive commercial glass dilators. A very easier and effective technique is to hold the dilator in place with a tight garment, maintaining pressure by sitting on a running bicycle seat (mounted on a special stool or even a bicycle) [28]. The Vecchieti operation involves applying a traction device either transabdominally or by Laparoscopy [29]. Post operative traction creates a functional vagina in 7-9days. Those who are not willing for a
dilator technique in them neovagina is created surgically by Creastas modification of Williams vaginoplasty which is a quick and relatively simple [30 ]. Operative treatment should be reserved, where Franks method is unacceptable, or when well formed uterus is present, with fertility being preserved. Such patients get identified by retained menstrual blood. It is recommended to do an initial laparotomy to identify the cervical canal, if cervix is atretic, the uterus should be removed [31]. In simple imperforate hymen/transverse vaginal septum surgery is indicated. Even in complete vaginal agenesis most recommend against preserving fertility. The morbidity followin g surgery argues for the removal of mullertan structures, at the time of construction of a neovagina.

Transverse vaginal septum, is a failure of canalization of the distal third of the vagina and usually presents with obstruction and urinary frequency. Differential diagnosis is done from an imperforate hymen by lack of distension at the introitus by valselva manouevre. Transverse vaginal segment may be associated with abnormalities of the upper reproductive tract e.g. absence of segments of the fallopian tubes or unilateral absence of the fallopian tube and ovary [32].

Distal obstruction is the only malformation which has to be treated as an emergency, as any delay in the surgical treatment can lead to inflammatory changes and endometriosis. Hence a definitive surgery is warranted as soon as possible. No attempt at diagnostic needling should be done as it can convert a haematocolpos to a pyocolpos.

Although the patient is infertile, reassurance and support are necessary. Genetic offsprings can be achieved by collecting oocytes from the genetic mother, fertilization by the genetic father and placement of the embryo in a surrogate mother [33,34]. On analysis of 34 surrogacy offsprings resulting from oocytes retrieved from 58 women with congenital absence of uterus and vagina did not show any evidence of inheritance in a dominant fashion and thus making surrogate pregnancy a reasonable option for these disorders [35].

**Androgen Insensitivity (AI)**

Complete AI also known as testicular feminization, is the most likely diagnosis when a blind vaginal canal is encountered and uterus is absent. This is the third most common cause of primary amenorrhea, after mullerian agenesis and gonadal dysgenesis. The patient with AI is a male pseudohermaphrodite. The adjective sex refers to the gonadal sex; thus the individual is having a testis and a male karyotype of 46XY. Pseudohermaphroditism means that the genitalia are opposite of the gonads; hence the patient is phenotypically female but with absent or meager pubic and axillary hair. Male hermaphrodite is basically a genetic and gonadal male with failure of virilization. Transmission is by X linked recessive gene which is responsible for the androgen intracellular receptor. Clinically this diagnosis should be suspected in i) afemale
child having inguinal hernias, because the testis are generally partially descended. ii) a patient with primary amenorrhea and absent uterus iii) a patient with absent body hair. Most of these children appear normal except for the inguinal hernias and are mostly not seen until puberty. They have normal growth and development, although overall height is usually greater than average, with eunuchoidal tendencies (long hands, big arms and big feet). The breasts although large are abnormal; as actual glandular tissue is not abundant, with small nipples and pale areolae. Greater than 50% have an inguinal hernia, labia majora are usually underdeveloped and the blind vagina is less deep than normal. Rudimentary fallopian tubes are composed of fibromuscular tissue and only epithelial lining. Horseshoe kidneys have been reported in these patients. The testis may be intraabdominal but are usually in a hernia. They are similar to the cryptorchid testis, except that they may be nodular. After puberty testis display immature tubular development, with tubules lined by immature germ cells and sertoli cells. No spermatogenesis occurs. There is high incidence of neoplasia in these gonads. In a study of 50 cases studied there were 11 malignancies, 15 adenomas and 10 benign cysts, giving a 22% incidence of malignancy and a 52% incidence of neoplasia [35]. In a more recent series a lower overall incidence of gonadal tumours was found ranging from 5-10% [37,38-40]. Therefore once full development is attained at puberty, gonads should be removed, at approximately 16-18 years of age and patient should receive hormonal therapy. This is the only condition when gonads with a Y chromosome should be removed as soon as diagnosis is made. First reason is that the development achieved with hormone treatment does not match the smooth pubertal changes due to endogenous hormones and secondly gonadal tumors have not been encountered prior to puberty in these patients. Gonadal tissue can be removed laparoscopically and laparotomy is only indicated in patients whose gonads are inaccessible [41].

On initial diagnosis of this syndrome since 17ketosteroids were found to be normal, an androgen resistance was suggested as compared to absence of androgens. Actually plasma T are in the normal to high normal range, along with normal metabolism and clearance of T. Hence having normal T levels these patients do not respond either to endogenous or exogenously given androgens. Hence the critical step required for sexual differentiation, where androgens are required, does not take place and hence the development is totally female. Since AMH is present development of mullerian structures completely gets inhibited, leading to absence of uterus tubes and an upper vagina. Thus in this syndrome a combination of i) normal female phenotype ii) normal male karyotype of 46XY iii) Normal or elevated blood T levels along with high LH is present.

Incomplete AI occurs 1/10th of those number of AI patients, where individuals have some androgen effect. They may have a mild clitoral enlargement or have a phallus developed. In them axillary and pubic hair develop along with a breast development. Gonadectomy should
not be postponed as it will prevent further unwanted virilization.

Previously unwanted and unthinking disclosure of patient genetic sex was done. This has changed as more and more patients want to understand their problem themselves. Although infertile these patients are complete female in their gender identity which should be reinforced.

**Compartment II:- Disorders of the Ovary**

Gonadal development problems can present with either primary or secondary amenorrhea. From 30-40% of cases of primary amenorrhea cases have gonadal streaks due to abnormal gonadal development; known as gonadal dysgenesis. They are grouped according to the following karyotypes.

i) 50%-45X ii) 25% Mosaic iii) 25% 46XX

Women with GD can also present with secondary amenorrhea. The Karyotypes here in decreasing frequency are i) 46XX (Commonest) ii) Mosaics-45X/46XX iii) Deletions in the long or short arm of X chromosome iv) 47XXX v) 45X. Both X chromosomes are required to be present and active in oocytes to avoid accelerated loss of follicles. Finding a normal karyotyping is the most difficult to explain regarding ovarian failure, suggestions made are possibly due to specific gene alterations. Some believe there are specific genes confined to a portion of X chromosome, which are necessary for normal ovarian function [42].

GD is also linked to neurosensory deafness associated with a normal female karyotype known as Perrault Syndrome. Therefore auditory evaluation should be considered in all GD cases with 46XX Karyotype.

Pure GD indicates the presence of bilateral streak gonads irrespective of the karyotype. Mixed GD indicates testicular tissue on one side and streak gonad on the other.

**Turner Syndrome (TS)**

Characterized by short stature, webbed neck, shield chest, increased carrying angle of the elbow in combination with hypergonadotropic hypoestrogenic amenorrhea, which makes diagnosis easy just on superficial evaluation. Because of lack of ovarian follicles there are no hormones produced at puberty, hence patient presents with primary amenorrhea. There are some less common variants of the syndrome. Autoimmune disorders, cardiovascular abnormalities and various renal anomalies should be ruled out. A karyotype should be performed in all cases with elevated gonadotropins, despite the typical appearance of a case of TS. The presence of a pure syndrome, 45 X chromosome single line should be confirmed. The reason is that it is not a simple academic exercise but in 40% of individuals diagnosed to have TS one finds mosaicism or have a structural anomaly of the X or Y chromosomes.

**Mosaicism**

It is important to rule out multiple cell lines of varying chromosomes (mosaicism), because the presence of a Y chromosome in the gonadal area requires the exci-
sion of the gonad, because the presence of any medullary (testicular) component within the gonad predisposes to tumour formation, as well as heterosexual development leading to virilization. Only in complete AI can the gonadal removal be postponed till after puberty a patient is resistant to androgens and occurrence of tumors is late. In all other patients having a Y chromosome gonadectomy should be done as early as possible, both because of tumour formation and early virilization. 30% of patients having a Y chromosome may not develop virilization at all. Hence in patients with a silent Y chromosome karyotyping becomes all the more essential because of the risk of a gonadal tumour. The fully stained and banded karyotype remains the best to detect the presence of testicular tissue or other mosaic combinations.

Roughly 5% of women who have been diagnosed to have TS, have Y chromosome on their karyotype [43]. On further analyses with Y specific DNA probes, ne can find another 5% with chromosomal material [43,44]. Though development of a gonadoblastoma in TS patients is low (5-10%) and appears to be limited when Y chromosome material is apparent on routine karyotyping. Thus probing for a Y chromosome in a TS patient would be indicated when virilization occurs despite no apparent Y chromosome in the karyotype and when a chromosomal fragment of uncertain origin is identified.

Even in absence of a Y containing line the impact of mosaicism can be significant. If the patient has an XX component (XX/XO) the functional cortical tissue can be found within the gonad, which can lead to a variety of responses, which includes some degree of female development and occasionally even menses and reproduction. These individuals may appear normal with normal height till patient attains a premature menopause. Mostly these patients are short statural. Most patients with missing sex chromosome have a height of <63” (160cm). The menopause is early as the follicles undergo an accelerated rate of atresia. Occasionally even autosomal abnormalities may be associated with hypergonadotrophic ovarian failure as occurred in a 28year old lady presenting with secondary amenorrhea along with elevated gonadotropins and had a trisomy 18 mosaicism [45]. All patients having absent ovarian function and quantitative alterations in sex chromosomes are labeled under the heading gonadal dysgenesis.

Gonadal Dysgenesis

A female patient presenting with an XY karyotype, with palpable Mullerian structures, normal female T levels and lack of sexual development is known as Swyer’s Syndrome. Since tumour transformation can occur at any age, gonads should be removed as soon as diagnosis is made [46] on details of sry mutations and other causes of gonadal dysgenesis.

Gonadal Agenesis

There is no complicated associations and only one can put a conjecture on the aetiological factors, like viral and
metabolic in early gestation or an undiscovered genetic mutation can be made. Ultimate result is hypergonadotropic hypogonadism. Development is of a female. Gonadal streaks do need to be removed to avoid the chances of neoplasia.

**The Resistant Ovary Syndrome**

This represents a rare case of patient having amenorrhea with normal growth and development, elevated gonadotropins though unstimulated follicles are present, with no evidence of autoimmune disease. Laparotomy is required to arrive at the correct diagnosis, showing histologic presence of follicles and absence of lymphocytic infiltration, as seen in autoimmune diseases. Although diagnosis gets proved it s not worthwhile to do laparotomy just to prove that as these patients do not respond to the highest doses of gonadotropins. These patients are excellent candidates for oocyte donation. It is not known whether this is a separate syndrome or they just represent a category of P.O.F, in which case unresponsive follicles are similar to those seen around menopause.

**Premature Ovarian Failure (P.O.F)**

The early depletion of follicles is surprisingly common. Roughly 1% women will experience P.O.F before the age of 40, while in women with primary amenorrhea the prevalence ranges from 10%-28% [47-49].

The etiology of P.O.F is unknown in most case. But it is better to explain to the patient that it is possibly a genetic disorder with an increased risk of follicle disappearance. Specific sex anomalies can be identified often [50]. Commonest ones are 45X and 47XXY, followed by mosaicism and specific structural abnormalities on the sex chromosomes. On searching for 45X/46XX mosaicism using fluorescent in situ hybridization a larger percentage of cells containing single X can be detected in women who present with P.O.F [51]. Translocations in the critical region on the long arm of chromosome have been described in women with P.O.F [52,53]. Mechanism of ovarian failure is mostly due to accelerated follicular atresia as even in TS, patients begin with a full follicle complement of germ cells. P.O.F is more common in families which contain the fragile X syndrome, which is a relatively common cause of developmental disability, which suggests that it would be useful to screen for fragile X syndrome in families with a history of P.O.F [54]. It is important to understand that carriers of fragile X syndrome are at an increased risk of P.O.F but no other medical problem [55]. Blepharophimosis/ptosis/epicanthus inversus syndrome which is an autosomal dominant condition has been associated with eyelid abnormality and P.O.F, which is caused by mutations in a transcription factor gene FOXL2 on chromosome3 [56]. Besides that P.O.F, can be due to an autoimmune process or perhaps destruction of follicles by infections like mumps, oophoritis or a physical insult like irradiation or radiotherapy.
The problem can present at varying ages on the basis of number of follicles left. If loss of follicles is rapid then primary amenorrhea and lack of sexual development will be present. In case loss of follicles occurs after puberty, then the extent of adult phenotypic development and time of onset of secondary amenorrhea will vary accordingly. Although many cases resume spontaneous menses with a normal karyotype this does not warrant a full thickness ovarian biopsy. Minimal approach i.e. recommended, with no definitive method to rule out autoimmune disease and checking the O-P activity. As far as hormonal therapy is concerned in view of hypogonadal patients in view of spontaneous ovulations with E-P contraceptive therapy, that is the treatment of choice. Best chance of pregnancy is with donor oocytes; important to note that with siblings's oocytes pregnancy rates are reduced [57]. Idiopathic P.O.F with corticosteroids is not warranted as responsiveness to gonadotropins is not achieved [58].

Molecular Explanation for Ovarian Failure

In a group of patients normal chromosome pattern has been identified with P.O.F in Finland displaying an recessive inheritance pattern [59]. In this population a point mutation of FSH Receptor was demonstrated to be the cause of ovarian failure in this population [60]. It accounted for 29% of 75 Finnish women presenting with P.O.F, with a general prevalence of 0.96 [61]. Ovarian follicles were present in these ovaries although ovaries were small on USG. A large number of inherited conditions are present in Finland. Trying to search the same mutation in the US, Brazil, Switzerland, Denmark, Japan and Singapore could detect only a single case in Switzerland in patients presenting with P.O.F [61-64]. In Finland, other specific mutations in the FSH receptor gene have been identified, but they remain very rare causes of P.O.F [65]. However it should be expected that an occasional patient of P.O.F will have mutation of the FSH receptor gene [66-68]. The management does not get much affected by documenting a specific mutation, or genetic cause of hypogonadism.

As more and more patients are investigated for genetic studies, multiple subgroups, stand to be identified, each with a different biological cause of ovarian failure, e.g a case of hypergonadotrophic primary amenorrhea has been reported due to point mutation in the LH receptor gene; FSH and LH were only mildly elevated with multiple ovarian follicles present with development and steroidogenesis up to early antral stage [69]. The reason gonadotropins are almost within normal range is due to inhibition by inhibin, as inhibin secretion by granulose cells is FSH dependent, not being influenced by LH. This same patient had two siblings who were 46XY male pseudohermaphroditism because of same LH-receptor mutation. In another example translocations on regions on X and Y chromosome which share sequence homology, have been reported with ovarian failure [70]. Sequences on the long arm of X chromosome (Xq27-28) share homology with long arm of the Y chromosome
(Yq11.22) allowing errors in the process of crossing over. Besides that, less than complete mutations also known as permutations of the site that transmits the fragile X syndrome have been reported to occur in a greater frequency in women with P.O.F [71]. Deletions of the X chromosome are rare in secondary amenorrhea, but occasionally a deletion can be detected in women with a familial history of P.O.F. [72].

The Effect of Radiation and Chemotherapy

Effect of radiation is dependent on both the age as well as the xray dose [73,74]. The gonadotropins rise and steroid levels begin to fall within 2weeks of irradiation to ovaries. The greater number of oocytes in a younger age is responsible for the resistance to total castration in young women exposed to intense radiation. After many years of amenorrhea the function may resume. Conversely, the damage may not appear until later in the form of P.O.F. Once pregnancy does occur the risk of congenital malformations is no greater than normal. If the irradiation field excludes the pelvis there is no risk of P.O.F. [75]. That is why elective transposition by laparoscopy out of the pelvis prior to irradiation provides a good prospect for future fertility [76]. Gonads are not in danger in the kitchen; microwave ovens utilize wavelengths with low tissue penetrating power. Doses that cause sterilization are as follows—with an ovarian dose of 60rads—no effect is noted, with 150 rads some risk is there over the age of 40, at 250-500rads, there is 60% chance of sterilization in ages 15-40, with 500-800rads the chance increase to 60-70% getting sterilized in ages 15-40 and over 800rads 100% get sterilized permanently [77].

Alkylation agents are very toxic to the gonads. As with radiation, there is an inverse relationship between the dose required for ovarian failure and age at start of therapy [78]. Other chemotherapeutic agents have the potential for ovarian damage but they have been less studied. The effect of combination chemotherapies is similar to alkylating agents. Approximately 2/3rd of women with breast cancer and treated with cyclophosphamide, 5fluorouracil and methotrexate lose ovarian function [79]. Resumption of menses and pregnancy can occur, but there is no way to predict which patient will reacquire ovulatory function [80].

In a study conducted by Valentinna et al in a multicentric survey of 1954 women with a BRCA1 or BRCA2 mutation where they studied women treated with invasive breast cancer between 26-47 years. Of the 1426 women receiving chemotherapy 35% experienced long term amenorrhea. Of the 528 women who did not receive chemotherapy 5.3% developed this kind of amenorrhea. Probability of getting chemotherapy induced amenorrhea were 7.2%, for women who were diagnosed before the age of 30yrs, 33% for those diagnosed between 31-44yrs and 79% for those diagnosed after 45yrs of age. Also this was higher for women receiving tamoxifen. Thus they con-
cluded age at treatment and use of tamoxifen were important predictors of chemotherapy induced amenorrhea in women carrying a BRCA1/BRCA2 mutation. The risk of induced long term amenorrhea is not greater among mutation carriers than among women who did not carry any mutations [81]. Bah et al studying 33 women for the risk of low dose iv cyclophosphamide used for treatment for SLE, found that the risk of iv low dose cyclophosphamide inducing long term amenorrhea was minimal in patients <40yrs but high in women receiving the treatment >=40yrs [82].

There is a question posed that is it possible that if ovarian follicles are maintained in a dormant state by suppressing FSH secretion can prevent ovarian failure. In a monkey model, gonadotroph suppression by treatment with GnRH agonist during radiation did not protect the ovarian follicles [83]. But GnRH agonist treatment of monkeys did protect the ovarian follicles against damage by cyclophosphamide [84]. Limited experience with chemotherapy and GnRH agonist has been encouraging [85].

The harvesting and cryopreservation of oocytes prior to irradiation and/or chemotherapy, seems to be the ultimate best means of preserving fertility for these patients. The other possibility is transplantation of ovarian tissue into a peripheral site, a method that has yielded a successful pregnancy in a monkey [86].

Compartment III
Pituitary Causes
Hyperprolactinemia

Hyperprolactinemia is one of the most common causes of pituitary related amenorrhea, which may be physiologic or pathologic. Although the exact mechanism by which it causes hypogonadotropic hypogonadism is not clear; because these women have low LH pulse frequency and decreased LH responsiveness to estrogen [87], it is proposed that GnRH suppression maybe a key factor. Treatment of hyperprolactinemic amenorrheic women with pulsatile GnRH secretion causes follicular maturation and ovulation when coupled with an HCG trigger, which supports this above hypothesis [87]. Besides CRH and Kp may be important mediators of GnRH suppression. In rodents prolactin–receptor mRNA has been localized to kp neurons in the hypothalamus [88] and administration of kp to hyperprolactinaemic female mice increase circulating gonadotropin levels and restores ovulation [89]. Since in vitro prolactin disrupts granulosa cell function [90], prolactin may also be directly disrupting ovarian function.

Physiological Causes of Hyperprolactinemia
Pregnancy

Pregnancy is the commonest cause. Prolactin levels increase during pregnancy, peak during delivery-levels
reach as high as 600ng/ml [91]. In nonnursing women, prolactin levels decrease during first 72h postpartum [91,92] and normalize in 1st three weeks [92].

**Lactation**

Suckling increases prolactin levels, which subsides by 12weeks postpartum, following drop in E2 levels, which leads to decrease in lactotroph hyperplasia [91]. Cause of lactational amenorrhea is dependent on factors like maternal nutritional status in relative to frequency and intensity of nursing [93] and hyperprolactinemia. All factors like prolactin levels, their response to suckling as well as prolactin bioactivity are associated with duration of amenorrhea [94-96], lactational amenorrhea is also associated with decreased LH pulse amplitude and frequency [97], along with reduced responsiveness to estrogen [98]. Pulsatile GnRH administration stimulates follicle maturation, ovulation and luteal phase function [97], which suggests that GnRH suppression maybe a causative factor.

**Macroprolactinemia**

The predominant form of prolactin is a 23Kd form, but in some individuals, a significant form of circulating prolactin consists of higher molecular weight form known as macroprolactin, which is thought to be a complex of 23Kd form and an immunoglobulin [99], or a complex consisting of glycosylated proteins [100]. This is cleared more slowly from the circulation than the 23Kd aggregates [101] and is less bioactive, which supports macroprolactinemia despite hyperprolactinemia being asymptomatic [102]. Upto25% of individuals with hyperprolactinemia may have macroprolactinemia [103,104], hence it is essential to detect these macroprolactin to prevent unnecessary treatment. Its detection can be done by gel filtration chromatography or polyethylene precipitation [105].

**Pathophysiological Causes of Hyperprolactinemia**

**Lactotroph Adenoma**

Prolactin secreting adenomas are the commonest secretory pituitary adenoma subtypes. They are usually benign and divided into micro and macroprolactinomas according to tumour size being <1cm and greater, along with prolactin levels <=100ng/ml and >1000ng/ml respectively. Dopamine agonists remain the treatment of choice in prolactin secreting adenomas be it bromocriptine with whom the most experience had been gathered or more effective cabergoline which has now been tested in pregnancy with not significant side effects or any significant congenital malformations [106,107]. Individuals with large adenomas may have serum prolactin around 1000ng/ml, yet with poorly differentiated or cystic lesions [108] and prolactin levels will be lower than expected based on size.

ii) Stalk disruption-Arcuate nucleus neurons of hypothalamus produces dopamine which tonically suppresses pituitary prolactin production. Hence if there is disruption of stalk connecting pituitary to hypothalamus flow of
dopamine to pituitary gland is prevented which ultimately results in hyperprolactinemia and amenorrhoea. Similarly large sellar masses or traumatic injuries lead to stalk disruption.

**Primary Hypothyroidism**

Normally TRH which is secreted from the hypothalamus stimulates not only the TSH release from the anterior pituitary but also simultaneous secretion of prolactin [109]. Primary hypothyroidism patients have greater prolactin response to TRH, which leads to greater prolactin secretion in response to TRH [110], which results in hyperprolactinemia with resultant amenorrhea [111]. Similarly significant enlargement of pituitary gland secondary to thyroid hyperplasia and possibly lactotroph hyperplasia may occur in primary hyperthyroidism, which regresses subsequent to treatment [112], along with normalization of the raised prolactin levels [113].

**Chronic Renal Failure**

Hyperprolactinemia results secondary to both decreased clearance along with increased secretion of prolactin in patients with chronic renal failure. Increased prolactin secretion may occur due to decreased responsiveness of lactotrophs to dopamine suppression [114].

**Chest wall injury**-Rarely chest wall injury causes hyperprolactinemia [115]. If there is chest wall burns of severe degree, intercostal nerves blockade caused normalization of serum prolactin levels [115], which suggests that a neurogenic stimulus at the site of injury is responsible.

**Non Lactotroph Adenomas and Sellar Masses**

Amenorrhea can be caused by any sellar masses. This occurs secondary to either hyperprolactinemia due to stalk disruption and/or compression of pituitary gonadotrophins, especially when lesion is >=1cm. Hormonal effects may also be causative in amenorrhea development in functioning adenomas.

**Cushing’s Disease**

ACTH secreting adenomas (Cushing’s disease) commonly causes amenorrhea. In 45 cases of Cushing’s disease studied, 33% were amenorrheic, with amenorrhea associated with greater mean serum cortisol, lower E2 and SHBG levels [116]. Though adrenal androgens are raised in Cushing’s disease, amenorrhea was not associated with increased serum androgen levels or free androgen index [116]. Hence amenorrhea is more likely caused by suppression of GnRH by cortisol [116], than by hyerandrogenism.

**Acromegaly**

Even in GH secreting adenomas as well as acromegaly amenorrhea and resulting infertility is common. In 47 women studied with acromegaly, 62% of them had amenorrhea, associated with higher GH levels, along with lower LH and E2 levels [50]. Compression of pituitary gonado-
trophs—either due to cosecretion of prolactin by stalk compression or by co-secretion of prolactin by tumour, maybe responsible for amenorrhea development, also decreased SHBG may result in decreased androgen bioavailability [117]. T in normal cycling women decreases LH pulse frequency which suggests increased androgen activity may be responsible for amenorrhea [118]. Treatment can be with octreotide or surgery.

**Thyrotroph adenoma**—TSH secreting adenomas with coexisting hyperthyroidism maybe associated with amenorrhea or oligomenorrhea and be the causative factor for amenorrhea. As compared to euthyroid women hyperthyroid women having amenorrhea have higher SHBG, FSH, LH and E2 levels [119], but do not have a midcycle LH peak, which suggests that amenorrhea is due to the failure of estrogen to stimulate LH release [120]. Treatment remains surgical option only.

**Infiltrative Processes**

An infectious disease/inflammatory process by infiltrating H-P-A axis can cause anterior pituitary dysfunction along with hypogonadotropic hypogonadism. From breast/lung tumour, metastasis may invade the H-P-A axis and cause amenorrhea [121].

**Hypophysitis**

Hypopituitarism results from the inflammatory infiltrate of the pituitary gland leading to hypophysitis, which results in hypopituitarism due to destruction of the cells in the anterior pituitary. This can also cause hyperprolactinemia due to stalk disruption, implying multiple mechanisms of amenorrhea. Lymphocytic and granulomatous hypophysitis are the 2 commonest types, which occurs primarily in females, gets commonly diagnosed during pregnancy or postpartum, with majority developing anterior pituitary dysfunction [122]. Granulomatous hypophysitis which is characterized by granulomas formed by multinucleated giant cells can occur in the setting of systemic diseases like Wegener’s granulomatosis [123]. IgG4 positive plasma cells rarely cause hypophysitis and amenorrhea though more seen in males [124] and leads to hypogonadotropic hypogonadism.

**Granulomatous Disease**

Tuberculosis and sarcoidosis may cause amenorrhea. While in sarcoidosis hypogonadotropic hypogonadism is due to granulomatous infiltration of hypothalamus, though granulomas can also invade pituitary and infiltrate gonadotroph cells [125]. Also both intersellar tuberculomas and tubercular meningitis have been associated with amenorrhea [126-128]. In a follow up study of 49 patients afflicted with tubercular meningitis in childhood, 10% developed gonadotropin deficiency, possibly due to progressive hypothalamic scarring [128].

Langherhans cell histiocytosis—Characterized by proliferation which is inappropriate followed by infiltration
of a type of dendritic cell into various body regions which includes H-P-A axis. Typically this is associated with diabetes insipidus but hypogonadotropic hypogonadism has been reported [129].

Sheehan’s Syndrome

The pituitary gland enlarges during pregnancy due to estrogen stimulation of lactotroph cells [130]. Superior hypophyseal artery may get compressed with the enlarging tissue, with this vessel being the major blood source to the anterior pituitary, which makes the gland vulnerable to changes in blood supply. Thus significant blood loss at peripartum period may lead to ischemic necrosis of the pituitary gland leading to hypopituitarism. This is known as Sheehan’s syndrome, which is usually seen in women having marked haemorrhage, although even with minimal postpartum bleeding it has been reported. Hence pituitary autoimmunity has been proposed to play a role in development of anterior pituitary function [131]. Improvements in management of peripartum haemodynamic complications, has significantly reduced the incidence of this disease in developed countries, although with recent reports it is suggested that incidence in developed countries may be greater than what was thought previously [132]. In France it was shown that a mean delay of years before diagnosis [133], which suggests that the diagnosis is overlooked in developed countries usually.

Traumatic Brain Injury

Mild head injuries or subarachnoid haemorrhage may result in amenorrhea and hypogonadism [134], especially those that require hospitalization. Sometimes the pituitary function loss may get delayed as much as 12 months from the initial event [135].

Iatrogenic Causes of Amenorrhea

Various drugs may act by
1. Inhibiting GnRH Release
2. Inducing hyperprolactinemia
3. Inducing hypophysitis
4. Inhibition of GnRH Release-Opioids

Opioids

Opioids is a common but under recognized cause of hypogonadism [136]. HH is a common side effect in men as compared to women [137]. In vitro as well as human studies show that opioids act by directly inhibiting GnRH release [138,139], although hyperprolactinemia may be an alternating cause for the hypogonadism [140].

Medication Induced Hyperprolactinemia

Psycotropics medications-The commonest ones are neuroleptics, acting by blocking dopamine receptors. Because of their having a greater affinity for the D2receptor [141], the typical antipsychotics have a more profound
effect on elevating prolactin levels [142]. Although there is conflicting data regarding selective serotonin reuptake inhibitors, in large open label study of depressed individuals it was found that 22% of women after using fluoxetine developed hyperprolactinemia. Similarly clomipramine, which is a tricyclic antidepressant, which inhibits serotonin uptake, also leads to increased prolactin levels [143]. Serotonin uptake inhibitors may be increasing prolactin levels by the serotonergic inhibition of dopamine neurons.

Other Medications—Certain GIT motility agents and antihypertensives by antidopaminergic mode of action cause hyperprolactinemia. Serum prolactin levels are 6 fold increased after oral or iv metoclopramide [144] and domperidone also stimulates prolactin release [145]. Methyldopa, which is an α-adrenergic agent and verapamil, a Ca channel blocker, both lead to increased prolactin levels, possibly by decreasing dopamine synthesis [146,147].

Medication Induced Hypophysitis

Cytotoxic T-Lymphocyteantigen 4 (CTLA4) Antibodies

Human monoclonal antibodies like ipilimumab and tremelimumab target CTLA 4 and thereby promote an immune mediated response against cancer. Ipilimumab has been approved by FDA to treat advanced malignant melanoma and is being studied in US in other malignancies. Due to its immunostimulatory properties ipilimumab is associated with autoimmune reactions, which include hypophysitis, which leads to hypopituitism. In a large randomized controlled trial in which ipilimumab in metastatic melanoma was studied the incidence of hypopituitarism was found to be 2.3% [148].

Pituitary Surgery

In contrast to the belief that surgical treatment causes hypogonadism, pituitary lesion is rarely associated with hypogonadism [148], it is an underestimate of the true prevalence. The reason is that surgeons with higher surgical volumes have fewer postsurgical complications [149], with most representing rates of hypogonadism of dedicated surgeons.

Radiation therapy given either to hypothalamus or pituitary is a known cause of hypopituitarism [150]. Even individuals who receive radiation therapy for nonpituitary tumours have a high risk of developing hypogonadism. Degree of hypogonadism and hyperprolactinemia in these patients depends on the dose of the radiation therapy given [151].

Compartment IV

Hypothalamic Causes

Functional Hypothalamic Amenorrhea

FHA is a very common cause of secondary amenorrhea [152]. This accounts for reproductive dysfunction seen in exercise, undernutrition, chronic disease and se-
vere emotional stress. In the face of nutritional or physical stress, it is adaptive for an organism to allocate energy resources for its survival rather than the costly process of reproduction and therefore FHA is an physiological response to environmental stress and physical stressors.

**Hormonal Mediators of FHA**

When physical, nutritional or extreme emotional stress occurs the HPA axis gets activated and inhibits the HPO axis at multiple levels. At the hypothalamic level, CRH suppresses GnRH secretion. In women who are cycling normally, an infusion of CRH suppresses FSH/LH secretion but this gets prevented by GnRH administration, which suggests that CRH acts by inhibiting GnRH secretion [153]. ii) At pituitary level, ACTH is shown to have negative reproductive effects in mice. In mice who have been adrenalectomized and maintained on physiological levels of glucocorticoids, ACTH administration x10 days results in absence of corpus lutea [154], which demonstrates that ACTH has reproductive effects independent of adrenal steroid production.

Also females with FHA have greater 24h mean plasma cortisol levels in contrast to controls [155] and cortisol which is the end product of the HPA Axis suppresses reproductive function at the hypothalamic, pituitary and uterine levels. i) In rhesus monkeys supraphysiological doses of hydrocortisone suppresses gonadotropin secretion, which is almost completely reversed by intermittent GnRH infusion, which suggests that cortisol suppresses GnRH secretion [156]. ii) Similarly women with Cushing’s disease and women on long term supraphysiological prednisolone therapy have a reduced LH response to GnRH [157,158], which suggests that glucocorticoids also suppress pituitary responsiveness of pituitary gonadotropins to hypothalamic input iii) Glucocorticoids also inhibit the effect of E2 on the uterus. Dexamethasone coadministered with E2 attenuates the expected increase in uterine weight seen with E2 alone, at least partly by reducing estrogen receptor concentrations [159]. Therefore crosstalk between the HPA and HPO axis promotes the development of amenorrhea as a functional adaptation to stress.

**Leptin**

Leptin levels are low in women with FHA [160,161], which is a hormone secreted by the adipocytes and this maybe a mediator of amenorrhea. Two studies investigated the effects of recombinant methionyl human leptin (rhleptin). In FHA women-in the first open label study, it was found that LH frequency increased 2 weeks post treatment with rhleptin [162] and in second, which was a randomized placebo controlled study, a significant number of women who were treated with rhleptin got a menstrual bleed, out of which half were associated with ovulation [163]. Though the exact mechanism of action by which a decrease in leptin cause anovulation is unknown, low leptin levels may suppress GnRH through akisspeptin (kp)-mediated pathway [164].
Insulin

Body weight changes be it weight gain or loss can affect fertility. Marked weight loss secondary to chronic undernutrition can cause FHA and is associated with low insulin levels, while appreciable weight gain may cause obesity associated with insulin resistance i.e. a state of functional hypoinsulinemia. In animals, insulin is shown to modulate HPO axis and therefore low or functionally low insulin levels may mediate infertility. Disruption of insulin receptors in a mouse model is associated with significantly reduced antral follicle count due to 90% reduction in circulating LH [165].

Fibroblast Growth Factor-21 (FGF21)

FGF21 is a liver derived hormone, whose production is upregulated in response to starvation [166,167]. FGF21 has been shown to be responsible for starvation induced amenorrhea [168]. FGF21 transgene mice are anovulatory, with low LH levels, while administration of GnRH but not gonadotrophins, elicit an ovulatory LH surge, which demonstrates that FGF21 acts at the level of hypothalamus to disrupt ovulation [168]. FGF21 transgenic mice have decreased kiss-1 gene expression in the antral periventricular nuclei of the hypothalamus and the product of Kiss-1, Kisspeptin is a potent stimulator of GnRH secretion, which suggests a mechanism by which FSH disrupts ovulation [168]. It is not clear whether FGF21 is a mediator of FHA in humans.

Adrenal Tumours

In glucocorticoid and/or androgen secreting adrenal neoplasms amenorrhea frequently occurs. The increased cortisol levels produced by glucocorticoid secreting tumours, probably suppress GnRH secretion leading to amenorrhea [156]. Those that produce androgens are usually malignant (53%) [169]. They produce symptoms like hirsutism, acne and amenorrhea [169], but very rare. Normally with normal menstrual cycles T decreases LH pulse frequency, which explains why oligomenorrhea occurs in such tumors.

Genetic Causes

Hypothalamic Idiopathic Hypogonadotropic Hypogonadism (IHH)

IHH comprises of a group of disorders with patients presenting with delayed or absent pubertal development, either due to a GnRH mutation or a GnRH deficiency, in which hypothalamic/pituitary imaging is normal. Usually it affects males, but can present in women as a cause of primary amenorrhea occasionally. The most common phenotypic association is anosmia in Kallmann’s syndrome (KS). Normally there is a shared embryonic development of GnRH neurons and olfactory neurons. Causes of IHH, may be due to mutation in KAL-1 gene [170], the FGF1 receptor [171], FGF8 [172], the CHD7 gene [173], gene encoding semaphorin 3A [174], semaphorin 7 [175], besides the genes encoding the prokineticin-2 and its re-
ceptor that is prokineticin receptor-2 [176]. Also genetic defects in GnRH secretion as well as function can be associated with normosmia, in IHH cases. Kisspeptin being a known regulator of GnRH secretion, mutations of genes like KISS1 and KISS-1R, which encode kp and its receptor, lead to hypogonadism [177,178]. Similarly mutations of leptin [179], its receptor [180] along with mutations, in the prohormone convertase 1 gene lead to severe hypogonadotropic hypogonadism along with severe obesity [181]. Other causes like GnRH receptor mutations, lead to HH, which typically do not respond to usual doses of pulsatile GnRH administration [182,183]. Similarly mutations, in TAC3 and TAC3R which encode for neurokinin B [184] cause IHH. In addition genes like HESX3 [185], Axl [186], TSHZ1 [187] are newer additions as the causative factors of IHH. The identification of zinc finger homeodomain factor teashirt finger family member 1 (TSHZ1), a key regulator of mammalian olfactory bulb (OB) development not only in mice but also in humans as revealed by Ragancokoya et al [187] has given some answers to a lot of unanswered questions regarding role of PROK2 ligands acting through their G protein coupled receptors PROKR2 regarding how their mutations cause both KS and IHH [188-191]. Although HESX3 has been another gene its mutations have currently been seen only in male cases of KS. Axl is another gene found to be involved in the migration of GnRH neurons.

In a study of 55 women with FHA, 13% were found to be having heterozygous mutations, in genes associated with IHH, as compared to no mutations in 422 controls [184]. Thus FHA results from an interplay between environmental as well as genetic factors, where inherited defects in GnRH biology may lower the threshold at which external stressors suppress the HPO axis [192].

Pituitary

Mutations in genes encoding transcription factors involved in the cellular proliferation and differentiation of the pituitary gland e.g. HESX1 [193] / GLI2 [194] and SOX3 [195] mutations, have been shown to be associated with hypopituitarism, while mutations in SOX2 [196], LHX3 [197], LHX4 [198] and PROP1 [199], may be responsible for gonadotropin deficiency. Other associated factors with the mutations may present with other clinical features helping in the diagnosis. An e.g. of this is SOX2 mutations may result in anophthalmia, while mutations in LHX3 have been known to be associated with a rotationally limited cervical spine [197].

Shaw et al retrospectively studied 248 female patients of IHH from 1980 to 2010 seen in Massachusetts general hospital. The clinical presentation varied from primary amenorrhea and absence of any sexual characteristics to spontaneous breast development and occasional menses. In this cohort rare sequence variants were present in all known genes associated with GnRH deficiency, including
novel identification of GnRH deficient women with KAL 1 variants. They concluded that the pathogenic mechanism through which KAL1 variant disrupts female reproductive development requires further investigation [200].

Management of HA

IHH in women (as in men) is treated with sex hormones. Initially low dose estradiol (1mg) is begun in order to develop sexual characteristics and then it is gradually increased in doses [201-204]. From the second year of treatment estrogen supplementation with chlormadinone acetate is done [202-204]. Desire for pregnancy warrants pulsatile GnRH therapy to stimulate production of serum FSHand LH [205,206]. Further the importance of GnRH administration should be intermittent and pulsatile to be able to restore activity of the reproductive axis in patients with HA and other disorders of GnRH deficiency was emphasized by Knobil et al [207]. Tonic exposure of GnRH inhibited pituitary gonadotropin secretion paradoxically [208]. Although chances of success is good, where GnRH is not available, LH or FSH can be administered alternatively [209].

Other causes like GnRH receptor mutations, lead to HH, which typically do not respond to usual doses of pulsatile GnRH administration, though successful conception with high dose pulsatile GnRH has been reported [210].

Just like in male subjects Abel et al repeated the study in 37 IHH women treated with i/v pulsatile GnRH therapy (75ng/kg/bolus)(retrospective study (1980-2012), all patients over 16yrs with 46% anosmic and tested for all 14 genes and found that during first cycle 60% (22/37) recreated normal cycles, 30% (12/37) demonstrated altered gonadotropin response indicating pituitary resistance and 10% (3/37) an exaggerated FSH response consistent with ovarian resistance. Mutations in CHD7, FGFR1, KAL1, TAC3, TACR3 were documented in IHH women with normal cycles, whereas mutations were identified in GNRHR, PROKR2 and FGFR1 in those with pituitary resistance. Women with ovarian resistance were mutation negative. Thus they concluded that although physiological replacement with GnRH recreates normal menstrual dynamics in most IHH ladies [211], Hypogonadotropic responses in first week of treatment identify a subset of women with pituitary dysfunction, only some of whom have GNRHR mutations. IHH women with hypergonadotropic responses to GnRH replacement, consistent with an additional ovarian defect did not have mutations in genes known to cause IHH similar to their findings in men with evidence of an additional testicular defect. Hence they hypothesized that identification of women with abnormal responses to physiological GnRH replacement would give greater insight into the pathogenesis of HH. Hyper/hypogonadotropic responses would implicate an ovarian/pituitary defect, respectively. Such findings would suggest that genes involved in gonadotropin or ovarian development
and function that are also expressed in the hypothalamus
should be given greater consideration in the search for
new IHH genes.

High dose kisspeptin 54 (kp-54) acutely stimulates
LH secretion in women of hypothalamic amenorrhea
(HA) but chronic administration is associated with desen-
sitization [212,213, 214]. Since GnRH has paradoxical
effects on reproductive activity Jayasena et al hypothesized
that their maybe a dose dependent therapeutic window
with in which kp treatment restores the GnRH/LH pul-
satility in a woman with HA. They examined 5 patients
with HA, with each having 6 assessments of LH pulsatility.
Single blinded continuous iv infusion of vehicle or kp54
(0.01, 0.03, 0.1, 0.3, 1nmol/kg/h) was administered. LH
pulses were monitored subsequently.

Kp restored LH Pulsatility in all patients with HA,
with peak responses observed at different doses in each
patient. The mean peak number of pulses during kp 54
infusion was 3 fold higher as compared to vehicle(number
of LH pulses/8h; 1.6+-0.4, vehicle;and 5+-0.5 with kp54,
p<0.01 vs vehicle). The mean peak LH pulse secretory
mass during kp54 was 6 fold higherLH as compared to
vehicle (pulse secretory mass in iu/l3.92+-2.31 for vehicle;
and 23.44+-12.59 with kp54 p<0.05 vs vehicle). Hence they
concluded that kp54 infusion temporarily increase LH
pulsatility in women with HA. They also determined the
dose range within which kp54 treatment increases basal
and pulsatile LH secretion in women having HA. Thus
this forms the basis for studying the potential of kp based
therapies to treat women with HA [215]. Since a distinct
combination of participants such as leptin deficiency,
weight loss, exercise, stress and mutations of GnRH asso-
ciated genes cause HA, in each of the 5 participants, etio-
logical differences may have accounted for heterogeneity
of individual response to kp54 administration [215].

Conclusion

Amenorrhea can present as PA or SA.In case of PA a
full thorough general physical eination, including the sec-
ondary sex characters comprising of breast development,
axillary and pubic hair, development of vagina, uterus and
ovaries by per rectum examination along with ultrasonog-
raphy to confirm the presence or absence of uterusand
vagina, rule out any inguinal hernias is important which
is then followed by a serum TSH, Prolactin and proges-
tional challenge test to rule out any hypothyroidism
or hyperprolactinemia,and a positive withdrawl bleed
clinches the presence of endogenous estrogen availabil-
ity. A thorough history is very important in case of ruling
out any end organ failure like Ashermann’s syndrome, or
for functional hypothalamic amemorrhea with history of
excessive exercise, eating disorders, competitive athletes,
dancers where excessive weight loss, or energy expendi-
ture leads to a reduction in body fat and BMI. Subsequent
investigations regarding karyotyping may be necessary
in a case of PA to rule out the presence or absence of Y
chromosomes in view of risk of development of gonadal
tumours. Genetic disorders are rare causes of PA presenting as KS/nIHH.S. gonadotrophins decide whether it is a hypogonadotropic hypogonadism or hypergonadotropic amenorrhea resulting in P.O.F., where further karyotyping is required to find the cause like mosaicism, turners, presence or absence of Y chromosome, or if normal karyotype for any FSH/LH receptor mutations. In case of hyperprolactinemia, depending on presence or absence of hypothyroidism, and level of serum prolactin (>100ng/ml) a MRI is required to rule out a microadenoma or macroadenoma which are treatable using dopamine agonists. Rarely hypophysitis and other causes of hypopituitarism may be present in case of SA. Detailed eating history, history of stress, exercise or strenuous activity may clinch the diagnosis of hypothalamic amenorrhea and management may be simple improvement of calories or reduction in energy expenditure which helps in restoration of LH pulsatility.

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