Section A -Research paper

Identification by cytogenetic analysis the Causative agents and treatment of Primary and Secondary Amenorrhea - A clinical review

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ABSTRACT

Amenorrhea is the abnormal cessation or absence of menstrual cycle in females. Regular menstrual flow indicates functioning neuroendocrine-reproductive system and assures that Physiological changes of puberty are normal.Primary amenorrhea is confirmed when a female is not attained menarche at the age between 14-16 .It might happen due to various reasons like Hypoplastic uterus or absence of uterus, hormonal changes, changes in glands and genetic disorders. Secondary amenorrhea is confirmed when a normally menstruating female couldn't able to menstruate continuously for three to six months. It might due to various reasons like hormonal imbalance, Pregnancy or abnormal growth in uterus, Polycystic ovarian failure, obesity, over exercises, depression, Stress, poor nutrition etc. Amenorrhea may be due to hormonal imbalance, ovarian failure, chromosomal abnormalities etc. Primary and Secondary amenorrhea can be diagnosed by blood test, USG, Karyotyping, Chromosomal microarray, Sanger sequencing etc. This article explains the causes, treatment and diagnostics of primary and secondary amenorrhea.

KEYWORDS: Amenorrhea, Menstrual cycle, Primary amenorrhea and Secondary amenorrhea

1. Introduction

Puberty is the period where the reproduction is possible and also there will be the development of gametogenic and endocrinal functions. Puberty starts with the blossoming of breast (thelarche), increase in growth size, and menstruation in a predictable order (menarche). Adrenarche, or sexual hair growth, is unrelated to GnRH function and occurs most commonly between breast budding and rapid development, but it can occur at any point during puberty. Adrenarche is triggered by the secretion of dehydro epiandrostenedione.

Menarche and regular menstrual cycles require adequate endocrine axis function, which includes the hypothalamus, pituitary, and ovaries. Amenorrhea can be caused by a disruption in this axis. The level of primary dysfunction must be defined before the pathophysiology of amenorrhea can be determined [1, 2].

Menses is the shedding of a woman's uterine endometrial lining in every 28 days (commonly termed as the womb) [3]. The terms, menstrual period, menstrual cycle, period and menses are all used to describe the term menstruation. The menstrual cycle is the events that occur in a woman's body every month as she prepares for the potential of pregnancy. The initial day of a menstrual shedding is countered as first day of a menstrual cycle. The average duration of the cycle is 28 days long; however, cycles can occur anywhere between from 21 to 35 days. Amenorrhea is the absence of menstruation in a female between the ages of 12 and 49. Various studies states that the cause of Amenorrhea can be due to a number of factors. Some could be normal and others could be a symptom of a medical illness or a side effect of medication [4].

2. Pathophysiology

The menstrual cycle is an organized series of hormonal processes in a woman's body that encourages follicle growth in order to release an egg during ovulation process and prepare a place for implantation if fertilization occurs. The moist decidua of the endometrial lining of the uterus (which was ready to receive a fertilized egg) is shredded in a flow of menses and in preparation for the next cycle when an egg produced by the ovary remains unfertilized. The physiologic phases of the menstrual cycle are follicular, ovulatory and luteal. Each phase has its own hormonal secretory system. The target organs of these reproductive hormones (pituitary, hypothalamus, uterus and ovary,) can assist identify the illness process that causes amenorrhea in a patient.

3. Physical examination and Patients History

At the time of physical examination and enquiry of patient history, physicians must first enquire about the patient's age and which age the patient began to menstruate at puberty (menarche). The history and age of menarche information is crucial for distinguishing between primary and secondary amenorrhea. It must be primary amenorrhea if the patient was not menstruation at all. Secondary amenorrhea will be the situation in all other circumstances. To rule out the genetic cause or defects of primary amenorrhea, the most significant thing to determine is the patient's psychosocial age, and also their intellectual quotient (IQ). Following that, clinicians should enquire about other the growth developments of organs, such as breast bud development, because the absence or underdeveloped breast bud by the age of 13 to 14 years shows estradiol deficit. Physicians must find out the time range of the absence of menses in the normal menstruating female to rule out secondary amenorrhea. Pregnancy is one of the major causes of secondary amenorrhea, so it should be identified first and next enquire about previous Asherman syndrome procedures [5, 6].

Table 1: Physical examination of Amenorrhea

Primary Amenorrhea	Secondary Amenorrhea
Pubertal development evaluation • Height • Weight • growth chart (BMI)	General • Height • Weight • growth chart (BMI)
Blood Pressure • elevated in Cushing and PCOS Breast Development (may revealed galactorrhea)	Breast Exam
Turner's Syndrome evaluation • Shield chest • Widely spaced nipples • Low hairline • Webbed neck	Thyroid exam
Skin Examine • Hirsutism • acne • striae • ↑sed pigmentation • vitiligo	Skin Examine • Hirsutism • acne • striae • Alanthosis nigricans • thickness or thinness • easy bruisability
Pelvic examine • clitoral size • presence of cervix, ovaries, uterus • Intactness of hymen • Depth of vagina • presence of vaginal septum	Pelvic exam • Atrophy • Vaginal dryness

For premature ovarian failure, patient history like night sweats, hot flushes and sleep disturbance should be collected, as a history of radiation therapy and chemotherapy for neoplasm, as they also can cause ovarian failure in females. The Rotterdam criteria should be used to rule out polycystic ovary syndrome. For Kallman syndrome and pituitary adenoma, a test for vision and a sense of smell should be done. Antipsychotics is one of the most prevalent causes, that leads to the elevation of prolactin levels, this also leads to amenorrhea, thus a drug history is crucial [5].

The physical examination can be performed to find out the causes such as malnutrition or hepatomegaly (Table 1). The following inspection should be included such as

1. Measuring the patient's BMI, weight, and height to see if he or she has a chronic ailment.
2. To find out the malnutrition and anorexia nervosa, check your body mass index (BMI).
3. Examining your teeth for signs of deterioration
4. Look for calluses or bruising on the metacarpophalangeal joints.

To rule out hyperandrogenemia, examine for hair loss, or acne and hirsutism. A skin disorder called acanthosis nigricans can also be a sign of polycystic ovarian syndromes. Examining the breast development, pubic hair growth, and clitoral index in a woman with amenorrhea is an important factor in general physical examination. A standard chest examination can rule out Turner syndrome. A basic examination to check pregnancy and a vaginal examination to identify hematocolpos in an imperforate hymen are also recommended [5, 7, 8].

4. Types of Amenorrhea

Based on the occurrence before and after the first menstrual period, Amenorrhea can be classified into two types. They are Primary Amenorrhea and Secondary Amenorrhea (Table 1).

4.1. Primary Amenorrhea

Primary amenorrhea is the failure to start menstruation in the females by the age of 14 and lack of secondary sexual characteristics, or the absence of menarche by the age of 16 in the absence of secondary sexual characteristics growth and development. Secondary amenorrhea, is described as the absence of menses for more than six months for a normally menstruating female. Despite the fact that most of the causes of primary and secondary amenorrhea are similar, might their proportion differs. Hypothalamic illnesses causing hypogonadotropic hypogonadism, gonadal disorders generating hypergonadotropic hypogonadism, abnormalities in endocrine glands, and congenital utero–vaginal malformations are among the causes of primary amenorrhea (Figure 1, Table 2). Primary amenorrhea can result in delay in menstruation or halted puberty, or with normal pubertal development without menarche, depending on the underlying causes [9].

4.2. Secondary Amenorrhea

Secondary amenorrhea is known as the lack of menses or periods for more than three months following menarche in a female who have had a healthy menstruation period. Menstrual cycles could become moderately uneven in the initial stages of ovarian malfunction, leading to amenorrhea. Females pursue clinical help for amenorrhea because two main reasons: concern for losing their fertility as well as worries that amenorrhea is a manifestation of a severe primary disease..

5. Primary and secondary amenorrhea causes

Anatomic abnormalities in the genital tract, hypothalamic or pituitary might causes, ovarian insufficiency, endocrinopathies, and chronic oligo- or anovulation are the major common causes of primary and secondary amenorrhea.

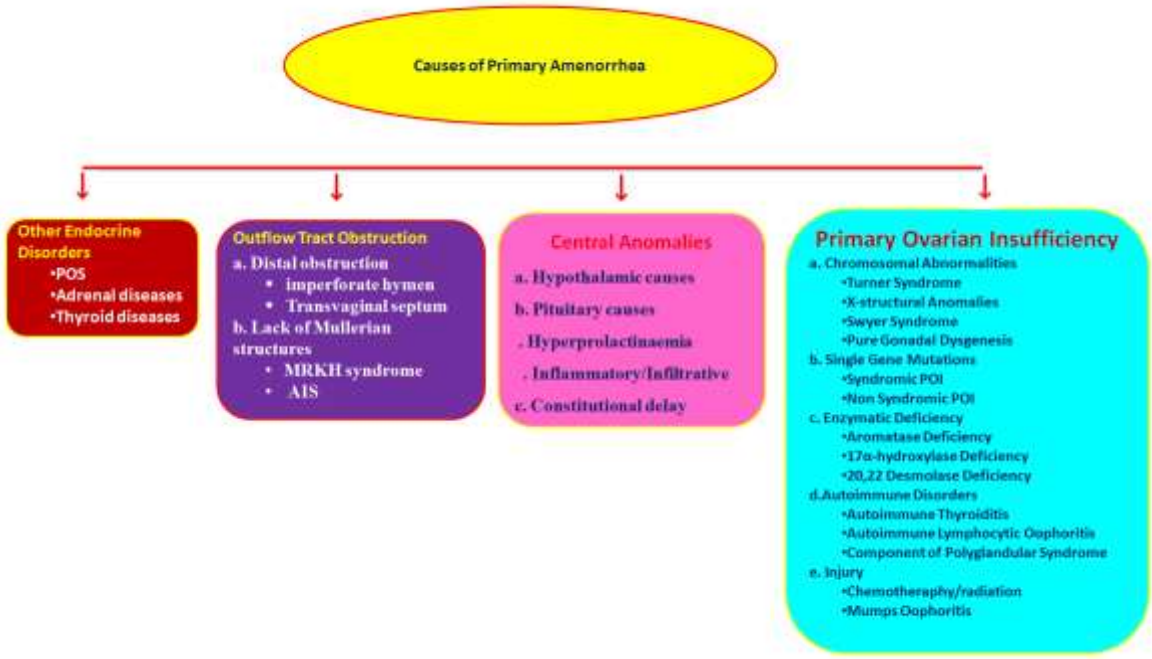

Figure 1: Cause of Primary Amenorrhea

5.1. Anatomic defects of the genital tract

Vaginal agenesis, transverse vaginal septum, imperforate hymen, cervical agenesis or dysgenesis, endometrial hypoplasia or aplasia, Mayer-Rokitansky-Küster-Hauser syndrome, and androgen insensitivity syndrome are all examples of anatomic genital malformations. Due to the anatomic barrier that prevents blood flow, vaginal agenesis should be suspected in all females with primary amenorrhea who experience frequent abdominal and pelvic pain. Furthermore, blood accumulation in the uterus (hematometra) can cause retrograde menstruation, which can progress to adherences and endometriosis [10].

A congenital vaginal blockage is represented by a transverse vaginal septum. It is stated that Transverse septum comes in two varieties, they are total variety and partial variety, but amenorrhea caused by total variant. The blockage might be found in the 16 percentage in the inferior vaginal region, 40 percentage in the central vaginal region and 46 percentage in the superior portion of the vaginal canal. This abnormality, like Agenesis of the vagina, in addition, this abnormality would be to account for persistent pain in the pelvic region or abdomen caused by deposition of blood within the vagina and the uterus. Imperforate hymen seems to be an uncommon congenital anomaly that manifests as amenorrhea, periodic stomach aches, and urinary obstruction in adolescent females. The overall frequency of imperforate hymen is considered to be one in a thousand female births. Since this illness is frequently asymptomatic, this is difficult to diagnose in children, while neonates might experience significant enlargement of abdomen in rare circumstances. Women having amenorrhea are more likely to be detected with an imperforate hymen later experiencing pain in abdomen, Hematometrium, in the course of pubertal phase [9, 11, 12].

Another common cause of primary amenorrhea is anatomical malformations in the cervix. Agenesis and dysgenesis are the two types of cervical abnormalities. Both of these problems can be linked to normal vaginal development. In particular, whereas incomplete development of cervical is seen in cervical agenesis or dysgenesis, patients with agenesis are

much more probably to introduce previously, patients who have had a background of primary amenorrhea seem to be more prone to develop with agenesis, extreme lower abdomen discomfort that occurs frequently in random periods. Hyperplasia of endometrium is a condition, refers to the incomplete maturation, thickens abnormally, proliferate abundantly, endometrium absent from birth. Traumatic intrauterine synechiae syndrome, or Ashermann syndrome or Intrauterine synechiae, is a relatively uncommon illness in teenagers, in reproductive ageing female, it is the most prominent source of serious secondary amenorrhea. When accompanied by symptoms including sterility, amenorrhea, miscarriage, is present, the condition is known as Asherman syndrome. The instance, miscarriage, PPH (postpartum haemorrhage) can all cause Asherman's syndrome, which can develop menstrual irregularities [9].

GRES (Genital Renal Ear Syndrome) also known as Mayer-Rokitansky-Küster-Hauser syndrome, CAUV (Congenital Absence of the Uterus and Vagina), seems to be a congenital female illness, with a frequency of 1/5.000. It is the most prevalent cause of amenorrhea with gonadal dysgenesis. Aplasia cutis congenital in the cervix and the vagina upper part in females with appropriate 46, XX karyotype and secondary sexual characteristics. The agenesis of Müllerian ducts derivatives distinguishes this disease, which is also known as "Müllerian agenesis". The major characteristics of MRKH syndrome include healthy ovaries, uterine defects varying in removal to basic uterine remnants, and aplasia of the vaginal upper part. The aetiology of MRKH syndrome remains unknown: while it was formerly thought that the disease was caused by sporadic abnormalities, a growing number of family instances have led to the assumption of a genetic base. In adolescents, regular secondary female features with primary amenorrhea is most all prevalent clinical manifestation. It is a tendency of serious lower stomach discomfort that has occurred on several occasions, just in few cases, where patients have rudimentary residues of uterus with a normal endometrial function, there is a history of recurring severe lower abdominal pain; furthermore, some adolescents can suffer psychological distress from unsuccessful sexual life. The basic concentrations estradiol and gonadotropin in plasma are normal, and there are no biochemical indicators of androgen overload, according to the endocrine examination [13, 14,15].

Androgen insensitivity syndrome (AIS) otherwise called a Testicular feminization syndrome or Androgen receptor deficiency is a condition of sex development characterized by resistance in hormone as a result of androgen receptor malfunction or X-linked recessive androgen receptor deficiency. On Xq11-12 chromosome, the gene that causes the problem has been found. Approximately 30 percentage of mutations are caused by incidental aberrations. CAIS (Complete androgen insensitivity syndrome) is an Androgen insensitivity condition in which a cell's incapacity to react with androgens and also absence of functioning 46 XY androgen receptors in patients. Furthermore, such individuals have cryptorchidy, having genitalia located in the GI tract; gonad remains functioning, release dihydrotestosterone and testosterone appropriate quantities [16, 17, 18, 19, 20, 21, 22].

While primary amenorrhea and scanty or missing pubic and axillary hair are typical symptoms of total androgen insensitivity syndrome, females also could develop a hiatal hernia in the course of babyhood. Furthermore, because the estimated prevalence having full AIS, shown in 1 percentage to 2percentage in females with inguinal hernia, few experts are

proposing that every girl with inguinal masses have a karyotype analysis. Since this is uncommon among the persons under age of 20, the prevalence of cancer in the testes was reported 22 percent. Hormonal testing typically reveals increased concentrations of testosterone and LH in plasma, in often conjunction with elevated estradiol concentration [23, 24, 19, 25].

5.2. Hypothalamic causes

In teenagers, a disorder of the hypothalamus is an important source for amenorrhea. For instance, women having hypothalamic problems are more likely to develop chronic anovulation as a result of inadequate synthesis of gonadotropin releasing hormone, which results in reduced basal concentration of estradiol and gonadotropins. On the other hand, exogenous GnRH stimulation, Gonadotropin production is within normal limits. Hypothalamic amenorrhea is often caused by a defective hypothalamus, while it can also be caused by other illnesses such as an isolated gonadotropin deficiency, chronic diseases, infections and tumors in rare situations. Psychogenic stress, intense physical exercise, and nutritional abnormalities are all dysfunctional causes of hypothalamic amenorrhea. In fact, the exact processes whereby extreme strain, loss of weight significantly affects GnRH release currently unclear. Furthermore, decreased GnRH synthesis in these females could exhibit a variety of effects the secretion of LH, pulses, changing from low into high or normal [26, 27, 28]. Excessive concentrations of CRH hormone, that suppresses pulse generator of GnRH, appear to be stimulated by psychogenic stress.

Girls who engage in a lot of physical exercise are also more likely to have hypothalamic amenorrhea and brief luteine phases. The severe physical activity and reduced calorie intake required to maintain leanness cause these anomalies. Actuality, athletes regularly exhibit a large disparity between calorie consumption as well as actual expenditure of energy, specifically in sports in which a decreased body mass is required in performance and attractiveness.Athletes, in instance, have a threefold increased prevalence of amenorrhea than the overall population, with marathon runner predominating. A strange illness known as female athlete triad syndrome recently identified with the help of insufficient calorie consumption. Athletes often display many components of triad, which contains osteoporosis, amenorrhea and eating problems. As a result, all of these changes must regularly check in addition can make the initial assessment as well as enhance the standard of a woman's life who participates in sports activities [29, 30]. Isolated gonadotropin releasing hormone deficiency, such as Anosmic idiopathic hypogonadotropic hypogonadism (IHH) or Kallman syndrome (KS) is an uncommon cause of hypothalamic amenorrhea. With a frequency of 1:40000 females, 1:8000 males, the KS is a genetically diverse developmental condition distinguished with GnRH insufficiency and structural abnormalities in olfactory neurosensory cells [31, 32].

5.3. Primary Ovarian Insufficiency

Primary ovarian insufficiency is a defect marked depletion of follicles or abnormality or dysfunction of follicles, which leads to a progression of ovarian impairment. An abnormality or impairment, a quantity of FSH in the range of menopausal recommended this, women before the age of 40 with oligomenorrhea were detected twice, one month apart [33, 34]. Primary ovarian insufficiency otherwise called hypergonadotropic hypogonadism (female), primary ovarian failure or premature menopause, resistant ovary syndrome and

premature ovarian failure. It affects around 1% of female less than 40 years. Menopause that occurs at the age of 50 is not the same as this condition, due to ovarian function's lack of brief consistency as a result of ageing. Idiopathic instances account for more than 90 percentage of all cases that are not associated with this disorder, however it can be linked to infection like herpes zoster, Iatrogenic orgin, anticancer therapy and radiation therapy, infiltrative processes, chemotherapy, polyglandular autoimmune syndrome, empty sella turcica/empty sella syndrome, syndromic defects and isolated defects.

Fragile X syndrome (FXS), is an inherited genetic disorder, causes a variety of developmental disorders such as learning impairments, cognitive deficits, and intellectual impairments.The FMR1 gene has a premutation, is seen in 6 percentage of girls having POI and a normal female karyotype. Turner syndrome is characterized by small stature as well as varying ovarian function deficits in certain people. A karyotype diagnosis should be considered in all cases with low stature or amenorrhea. So patients must be investigated with a variety of systemic complications like congestive heart failure, renal structural anomalies, hypothyreosis, auditory impairment, due to the patient might need treatment of HGH and HRT.

Several X chromosomal genes have been linked to POF, including BMP15, FMR1, FMR2, POF1b and its gene location Xp11.2, Xq27.3, Xq28, and Xq21.1-q23.3. In actuality, X chromosome issues have been linked to Turner syndrome with early ovarian failure patients, as well as partial deletion of the short arm of the X chromosome or translocations between Xp22 and 2p21 and the existence of a extra chromosome X. Autosomal genes such as FSH receptor, LH receptor, Inhibin A, FOXL2, GALT, FSH beta variant, ELF2B2,4,5, POLG, NOGGIN, LH beta, AIRE have been related to early ovarian failure in the majority of cases [35, 36, 37, 38, 39].

5.4. Medications and therapies

Amenorrhea can be caused by Prophylactic devices, Depo Provera, hormonal contraceptives, and intrauterine contraception, as well as injectable contraceptives. The menstrual period, sometimes take a few months to resume and become normal when discontinuing these kind of contraceptive methods. Menstrual periods can be stopped by certain medications, such as Antipsychotics, antidepressants, Cancer chemotherapy, Blood pressure drugs and Allergy medications, can raise the hormone level that suppresses ovulation. Radiation treatments with or without chemostherapy for Hematological malignancies like Leukemia, Lymphoma, multiple Myeloma as well as cancer in breast and gynaecological malignancies (Cervical cancer, Ovarian cancer, Vaginal cancer, Vulval cancer), may damage estrogen-producing cells in Granulosa cells of the ovulatory follicle and female gamete cell in ovaries , leading to amenorrhea Menstrual irregularities could occur as a result, but it is usually transient, particularly in adolescent women [40].

5.5. Gynaecological conditions

Secondary amenorrhea is a common sign of hormone imbalances, particularly those that cause or arise from them. Polycystic ovarian syndrome is a kind of polycystic ovary syndrom**e** If a female's body generates excess androgens than normal level, she develops Polycystic ovarian syndrome. High amounts of androgens in the body also lead to the ovarien cyst production, preventing eggs from being released (ovulation). The majority of females suffering PCOS with amenorrhea or oligomenorrhea. Primary ovarian insufficiency

caused by Fragile X syndrome (FXPOI). The phrase FXPOI refers to a disorder When ovaries quit working earlier they reaches menopause, which can happen as early as age 40.

6. Causes of Secondary Amenorrhea

It's important to think about the fundamental components required for regular menstruation and how they might be involved in the problem while trying to figure out what's causing pathologic amenorrhea. A dysfunctional hypothalamus, pituitary, ovary, uterus, or outflow tract can cause secondary amenorrhea (Table 2).

Table 2: Etiopathology of Amenorrhea

Anatomic genital defects	Hypothalamic causes	Genetic causes	Pituitary causes	Ovary insufficiency	Endocrine diseases
Vaginal agenesis	Functional hypothalamic amenorrhea • Psychogenic stress • Intensive physical activity (Excessive exercise) • Nutritional disorders	Turner syndrome	Hyperprolactinemia	Gonadal agenesis	Adrenal diseases • 17aHydroxylase deficiency • 17,20Lyase deficiency • Aromatase deficiency
Transverse vaginal septum	Isolated gonadotropin deficit • Kallman syndrome • Idiopathic	Pure gonaldal agenesis	Tumours secreting Hormone • FSH • LH • GH • TSH • PRL • ACTH	Gonadal dysgenesis	Thyropathies
Vaginal and uterine aplasia	Hypogonadotropic Hypogonadism	Androgen insensitivity syndrome	Inflammatory and infiltrative disorders	Congenital thymic aplasia	Hypothyroidism
Imperforate hymen	Anorexia nervosa	Mixed gonadal dysgenesis	Empty sella •	Premature ovarian failure	Poorly controlled diabetes

Cervical agenesis or dysgenesis	Chronic diseases Infections	5-alpha reductase deficiency	Panhypopitutarism •	Galactosemia	Primary ovarian failure
Endometrial hypoplasia or aplasia			Benign pituitary adenoma •	Enzymatic deficits	Congenital adrenal hyperplasia
Intrauterine synechiae			hypopituitarism • Sheehan syndrome • Head trauma • neoplasm		Ovarian tumours
Mayer-RokitanskyKüster-Hauser syndrome					Constitutional delay
Asherman's syndrome					
Androgen insensitivity syndrome					
Cervivo vaginal atresisa					

6.1 Hypothalamus

Secondary amenorrhea is most commonly caused by hypothalamic amenorrhea, which accounts for about 60% of all instances. An abnormality in the frequency of pulsatile gonadotropin-releasing hormone (GnRH) production is often likely responsible. Hypothalamic amenorrhea is a detection of limitation, but it is apparent that it happens frequently in some populations, such as anorexia nervosa patients, intense exercisers, and patients under stress. The hypothalamus receives a variety of impulses around the body and sends out Morse code in the form of (GnRH) pulses, the exact sequence that is crucial for proper communication to pituitary, which synthesis FSH and LH. Reduced gonadotropin hormone like Luteinizing hormone and Follicle stimulating hormone output and secondary

amenorrhea can be caused by disruptions in Gonadotropin releasing hormone transmission to pituitary from the hypothalamus. Infiltrative hypothalamic illnesses, such as Langerhans cell histiocytosis, sarcoidosis, and lymphoma can also impair hypothalamic function. Amenorrhea is caused by a change in GnRH secretion in this case as well. These women generally have headaches or neurologic abnormalities in addition to their other symptoms.

6.2. Pituitary gland

The pituitary gland produces LH and FSH in response to GnRH signals. There are a number of disorders that damage the pituitary gland and prevent it from producing enough gonadotropin. Hyperprolactinemia is a frequent disorder, and drugs can induce small increases in prolactin levels. To establish the relationship between medication alterations and the beginning of an alteration in the menstrual pattern, a thorough medical history is required. Prolactin concentrations can be modestly elevated (usually not much than 10 ng/dL over normal) by nipple activation (suckling) or chest wall damage, but only a small alteration in prolactin can affect the monthly rhythm. While hypothyroidism can promote hyperprolactinemia, high prolactin values should also trigger a thyroid function test. Sheehan syndrome is caused by pituitary infarction with postpartum hemorrhage or extreme hypotension, and it can cause amenorrhea. In the pituitary gland, as in the hypothalamus, infiltrative processes occur. Amenorrhea develops when the pituitary gland's function is disrupted, resulting in reduced gonadotropin production, inflammatory processes include hemochromatosis, sarcoidosis and lymphocytic hypophysitis which influence the pituitary gland [41, 42].

6.3. Ovaries

Sudden primary ovarian insufficiency or premature ovarian failure formerly mentioned as “ovarian failure', POI occurs if menopause or spontaneous failure of ovarian activity develops before the age of 40. Polycystic ovary syndrome is just a hormonal imbalance in women. It is a main source of amenorrhea and it is distinguished by irregular, chronic, infrequent or prolonged menstrual cycles. It generally involves signs and symptoms of hyperandrogenism/hirsutism. Premature ovarian failure seems to be the most frequent endocrine condition in reproductive-age women, affecting 7 to 10 percentages of women. Excess weight and/or increasing hyperandrogenism symptoms, as well as the development of oligomenorrhea or amenorrhea, are prevalent. The signs and symptoms of amenorrhea and hyperandrogenism may be concealed, since many females take contraceptives orally and other hormonal contraception in delayed adolescence or young adulthood. Insulin resistance is common in females with PCOS whom is obese or overweight, in addition to monthly irregularities. Metabolic syndrome like type 2 diabetes mellitus, heart disease, stroke, fatty liver disease, and obstructive sleep apnea/ obstructive sleep apnea–hypopnea syndrome are all conditions that they are more likely to develop PCOS [43, 44].

6.4. Uterus

Asherman syndrome, which is characterized by endometrial fibrosis and an absence of regeneration, is a less prevalent cause of amenorrhea. Furthermore, therapeutic endometrial burning has been utilized to produce endometrial fibrosis, producing in the intended lengthy contraception and amenorrhea. Asherman syndrome can arise in women who have endometrial tuberculosis. Asherman syndrome is more likely to develop in women who undergo uterine equipment for the time of being delivered. The endometrial layer is

more sensitive to damage and shows a reduced capacity to recover when oestrogen levels are low at the time of birth [45, 46].

6.4.1. Intrauterine Synechiae or Asherman's Syndrome

Asherman's syndrome is a gynaecological uterine condition that is rare and acquired. The scar tissue, which borders the uterine walls bonds together, reducing the size of the uterine cavity. Intrauterine adhesions destroy the uterine cavity fully or partially, causing amenorrhea. An induced abortion or an excessive postpartum curettage accompanied by endometritis and intrauterine scarification are the most common causes of adhesions. Menstrual blood flow is poor or absent in most Asherman's syndrome patients, who also have severe cramping and stomach discomfort. Several patients may have significant discomfort due to disrupted menstruation, while others may not get any unusual changes in their menstrual period and any ache. The morphology and structure of the uterus are frequently imaged in order to identify Asherman's syndrome. A hysteroscope, which is placed into the uterus and displays a real-time picture of the uterine cavity, hysteroscope tool is the gold standard for detection. Sadly, most gynaecologists' clinics do not have hyperscopes on hand. Hysteroscopic surgery to remove the uterine wall scar tissue is the most effective procedure for Asherman's syndrome. The hysteroscope gives the clinician a magnifying and clear vision of the uterus, allowing for precise uterine adhesion cutting. The majority of hysteroscopic surgeries can be performed as an outpatient procedure. Extreme forms of Asherman's syndrome can be much more difficult to treat because the cavity could be totally obstructed or too small to permit the hysteroscope to be inserted within the cervix.

7. Functional hypothalamic amenorrhea (FHA)

Stress-induced anovulation or FHA are amongst the most frequent form of secondary amenorrhea, and it explains the reproductive failure shown in undernourishment, heavy exercise, extreme mental stress, and chronic illness. Inadequate pulsatile GnRH-gonadotropin releasing hormone synthesis is thought to have at the root of FHA. The oscillations of both LH and FSH are reduced when GnRH secretion is abnormal (FSH). As a result, there is no rise in the secretion of LH in the mid of the menstruation, improper follicular growth, subsequent anovulation, and decreased plasma estradiol (E2) levels [46]. FHA is characterized by amenorrhea, decreased level of hypoestrogenism, and low blood gonadotropin levels. It's also crucial to figure out what's causing these symptoms, which could be a stress factor like low weight, movement, or emotional tension. FHA is diagnosed through exclusion, thus it's crucial to rule out alternative reasons of amenorrhea. Treatment of the underlying aetiology of hypogonadotropic hypogonadism is the most suitable and successful management for FHA. It entails supplying the appropriate quantity of energy in the form of adequate calories, preventing from excessive sports, and decreasing stress, as well as the capacity to cope with it. The numerous complications of FHA can only be overcome if the therapy is effective [47, 48, 49].

8. Hyperprolactinemia

A most prevalent aetiology of pituitary-associated amenorrhea is hyperprolactinemia, which can have physiologic or pathophysiologic origins. Although the specific mechanism whereby hyperprolactinemia induces hypogonadotropic hypogonadism is unknown, hyperprolactinemic females exhibit lower Luteinizing hormone -pulse frequency and lower LH susceptibility to oestrogen, showing that gonadotropin-releasing hormone suppression is

a crucial component. Therapy with pulsatile GnRH, if combined along with a human chorionic gonadotropin stimulus, causes follicular development and ovulation in hyperprolactinemic, amenorrheic females [50]. Some females with hyperprolactinemia possess ovulatory menstrual periods, despite the fact that the majority of them are amenorrheic. Because these women may still be infertile resulting in a shortened luteal-phase abnormality, therapeutic hyperprolactinemia could be necessary even in women who menstruate regularly. Importantly, a variety of physiological reasons of hyperprolactinemia with amenorrhea, such as pregnancy, need not require treatment and must be ruled out before treatment begins [51].

9. Potential Etiologies of Primary Amenorrhea:

Hypogonadotropic hypogonadism, Constitutional delay of puberty and growth; also, self limited, delayed puberty, combined pituitary hormone deficiency are also the conditions of the potential etiologies of primary amenorrhea. Studies states that functional hypothalamic amenorrhea, which cause Anorexia nervosa, other eating disorders, excessive exercise, stress, psychological illness, and chronic disease are some of the conditions that can lead to anorexia (e.g. Celiac disease, inflammatory bowel disease). Congenital hypogonadotropic hypogonadism also cause Isolated congenital hypogonadotropic hypogonadism; GnRH deficit and anosmia (Kallmann syndrome) [9]. Also, the condition like syndromic congenital hypogonadotropic hypogonadism which is often multiple pituitary hormone deficiencies caused by some of the syndromes that includes CHARGE syndrome, Waardenburg syndrome, Hartsfield syndrome, congenital adrenal hypoplasia, 4H syndrome (hypomyelination, hypogonadotropic hypogonadism, hypodontia), septo-optic dysplasia, holoprosencephaly, encephalocele, Prader–Willi syndrome, Laurence Moon syndrome, Gordon Holmes syndrome, Bardet–Biedl syndrome. Studies states that pituitary gland or stalk damage can be caused due to Adenomas and other tumors (i.e., prolactinoma, Cushing's disease, germinoma, craniopharyngioma); cysts (Rathke's cleft cyst, arachnoid, dermoid, epidermoid, suprasellar cysts, mucocele), Infiltrative disorders (autoimmune hypophysitis, hemochromatosis, sarcoidosis, Langerhans cell histiocytosis, granulomatous diseases); surgery, trauma, pituitary apoplexy, vascular lesions, empty sella syndrome, radiation therapy [9].

Several findings revealed that medications or using of certain drugs also can be a condition to be a primary amenorrhea. The medications/drugs include anesthetics, anticonvulsants, antipsychotics, gastrointestinal motility agents (such as metoclopramide, domperidone, ranitidine), opiates; selective serotonin uptake inhibitors; Oral contraceptives, alcohol abuse, heroin, cocaine, marijuana, glucocorticoids (high dose), exogenous androgens (transgender care). Another condition in primary amenorrhea is Premature Ovarian Insufficiency. This is caused due to turner syndrome, gonadal dysgenesis 46,XX -e.g., mutations in steroidogenic genes (17 hydroxylase deficiency, aromatase deficiency), FSH receptor gene, gonadal dysgenesis 46,XY -e.g., mutations in steroidogenic genes, SOX9, SRY, WT1, NR5A1, LH receptor gene, other genetic etiologies; autoimmune oophoritis, polyglandular autoimmune syndrome, irradiation or surgery, chemotherapy, infection [9].

10. Amenorrhea in Polycystic Ovary Syndrome

PCOS or Stein-Leventhal syndrome or polycystic ovarian disease (PCOD) is now the highest prominent endocrine condition in women both having primary amenorrhea/secondary

amenorrhea, affecting 3 to15 percent of childbearing age females. A woman can be diagnosed with polycystic ovarian syndrome if she fits any two things from three criteria: biochemical marks- free testosterone, androgen index like dehydroepiandrosterone sulphate, androstenedione and clinical symptoms of hyperandorgenism; ovulatory dysfunction; ultrasound technology/ ultrasonography detection of morphological appearance of polycystic ovary [52]. Menstrual irregularities, such as amenorrhea or oligomenorrhea, are the most common symptoms, which are often accompanied by increasing acne and hirsutism. These muted impacts of the production of GnRH in hyperandrogenism, leading to elevated synthesis of Luteinizing hormone, cause both primary or secondary amenorrhea in PCOS [53]. Other neurotransmitters that impact LH activity include neurokinin B and dynorphins, which are involved in the pathophysiology of PCOS. Tests for dihydrotestosterone, testosterone total/ free, Dehydroepiandrosterone sulfate, dehydroepiandrosterone and androstenedione are recommended to detect abnormal androgens biochemically. Since testosterone largely binds to glycoprotein like SHBG (sex hormone binding globulin) with a strong affinity, subsequently, it is associated with reductions in PCOS patients has increased level of free testosterone [54]. The anti-mullerian hormone (AMH) is an important endocrine marker that can be used to evaluate ovarian reserve and it is somewhat higher in women with polycystic ovarian syndrome , because of a higher amount of antral follicles and a larger formation by antral follicle than in women in the absences of PCOS [55]. The detection of PCOS can be aided by measuring blood AMH concentrations, which has been proposed as a diagnostic assay [53, 56].

11. Amenorrhea in Eating Disorders

Around 68 percent of an instances, amenorrhea is linked to eating problems. Despite the fact that amenorrhea was eliminated from clinical diagnostic guidelines for anorexia nervosa (AN), Binge eating disorder (BED), or eating disorder and bulimia nervosa (BN) in the Diagnostic & Statistical Manual of Mental Disorders (DSM-5), Menstrual issues affected adolescents with eating disorders make about one-third of the adolescent female's population. Reduced body weight, extreme activity or exercise, strain, and dietary restrictions/control are all associated with eating disorders, finally in a poor energy balance which may lead to amenorrhea. Menstrual abnormalities were linked to premature beginning eating disorders in teenagers, implying a connection, but not a causal relationship [57, 58].

Cystic fibrosis patients who struggle with adequate diet and maintain a reduced body weight may not show the similar tendency to menstrual cycle abnormalities as those who suffer from eating disorders, therefore decreased weight is not the primary consequence of amenorrhea. Amenorrhea affects nearly 25 percentage of anorexia nervosa patients prior to severe loss of weight, and restart of menstrual periods does not always happen with adequate gaining weight [59]. Rapid dropping of weight may be a marker of amenorrhea with anorexia nervosa patients, although the underlying reason could be alterations of the hypothalamic-pituitary adrenal axis. Caloric restriction may depress the hypothalamic-pituitary axis, causing alterations in the synthesis and liberation of GnRH, this causes the pulsatile release of LH to prepubertal stages, leading to synthesis of pituitary hormone like LH and FSH to cessation. Ovulation does not take place, owing to a diminished secretion and release of LH and FSH, decreased level of oestrogen, and ovulation does not occur. Amenorrhea can be treated with dietary therapy and weight loss, it will not guarantee that menses will start again.

Bulimia nervosa affected women, on the other hand, correlated with anorexia nervosa affected women, they have a decreased frequency of secondary amenorrhea, Amenorrhea in BN is hypothesized to be induced by interruption of HPA related to behaviours regulating body weight, which is probably related to the decreased Body mass index (BMI) reported on women with AN [60, 61].

Changes in insulin levels may contribute to hormonal abnormalities as a result of binge eating in BN. Binge eating episodes are often heavy in carbohydrate, resulting in elevated blood sugar and, as a result, the increased insulin release. Increased insulin levels can lead to a rise in testosterone, which can impair follicular development and ovulation, resulting in menstrual disorder. Bulimia has also been linked to PCOS, which causes menstruation disruption due to insulin resistance-mediated testosterone elevations.Hormonal imbalances, low weight might induce reproductive problems, obesity, also, can be caused by hormonal abnormalities [61].

12. Amenorrhea in Athlete women

Recognizing the health merits and drawbacks of women participating in sports activities is becoming increasingly important**.** While adolescent female athletic participation increased, so made their appearance of such a spectrum of monthly abnormalities, ranging from minor delays to amenorrhea [62]. The female athlete triad (FAT) seems to be a health problem that attacks women and teenagers who participate in athletics activities and involves three interrelated and not consistent dysfunctions: As previously mentioned, menstrual abnormalities, lower bone mineral concentration, and lower energy capacity are all factors to consider. Menstrual disorder is caused by a lack of energy, and both have a direct impact on bone health. While these dysfunctions occur in a wide range of sports activities, the probability of getting menstruation abnormalities and the female athlete triad (FAT) appears to be higher in sports such as long-distance running, ballet, gymnastics and figure skating in which a specific lean weight is stressed [62].

The female teenage athlete might suffer a large loss of bone mineral density as a response of the GnRH pulse generator termination, with significant consequences related towards the reduction in oestrogen synthesis shown in FHA (functional hypothalamic amenorrhea). Adolescence seems to be a critical period for bone development; unfortunately, when the hypothalamic system is disrupted, bone mineral density is disrupted as well, resulting in a rise in stress fractures as well as fibrous dysplasia in teenage sports having amenorrhea [63]. Intriguingly, links have indeed been found between the lower bone strength as well as additional dietary components, including leptin, which has been shown to be altered in amenorrheic athletes, suggesting that reduction in bone mass observed could be an effect of maintenance under the negative energy equilibrium [63].

13. Primary Ovary Insufficiency

13.1. Chromosomal Abnormalities

13.1.1. Swyer syndrome

Swyer syndrome is a sex development disorder caused by a hereditary defect that affects sexual organ development. Persons having one chromosome X,Y which is generally seen in men, external genitalia of a female present at birth, as well as streak gonads (ovaries or testes), which are also called as undeveloped gonads [64]. Swyer syndrome affects the majority of people who are reared as girls. This disorder causes infertility in adolescents who

do not go through normal puberty. This is created by mutations in one or more genes in certain cases, although the reason is unexplained in several cases [64, 65]. The pattern of inheritance is determined by the relevant gene. The chromosomal results, clinical examination, and imaging procedures are used to diagnose this illness. To avoid cancer, the streak gonads are removed, and hormone replacement treatment is used from adolescence onward. Despite Swyer syndrome makes women infertile, using donated eggs, women might be possible to conceive [62, 66].

13.1.1.1. Signs and symptoms

The signs and symptoms of Swyner's syndrome may include 46, XY chromosomes in female external genitals, Puberty that is delayed or non-existent, Menstrual periods are not present (primary amenorrhea), Ovaries or testes that aren't fully matured (streak gonads), 46, XY chromosomes in female external genitals (Figure 2), Puberty that is delayed or non-existent, Menstrual periods are not present (primary amenorrhea), Ovaries or testes that aren't fully matured (streak gonads). Moreover, various study states that Swyer syndrome affects the majority of people who have been raised but also considers as a woman. A woman may not show signs and symptoms until they reach adolescence, while they haven't reached puberty or haven't started menstruating. Secondary sex characters, including developing breasts, Mons pubis, as well as a normal menstruation can all be aided by hormone replacement treatment. Swyer syndrome patients are infertile, however egg donation and artificial reproductive technology may help them conceive.

13.1.1.2. Treatment

Swyer syndrome treatment may vary depending upon the individual's distinctive characteristics. Some patients require surgery to restore their external genitalia as well as to construct and/or enlarge their vaginal openings. From adolescence onwards, hormone replacement therapy (HRT) is normally required, and oestrogen and progesterone are commonly used. HRT may help to reduce osteoporosis like thinning and bone loss in later years of life, aside from assisting with the natural secondary sex character development. Dysgenetic gonads/streak gonads (abnormally developed tests or ovaries) are a type of dysgenetic gonad seen in the abdomen, Swyer syndrome patients frequently experience these symptoms, are more likely to develop cancer in gonad or gonadoblastoma and it needs to be removed surgically. Despite the fact that females having Swyer syndrome remain sterile, egg donation can help them conceive and carry to term [67].

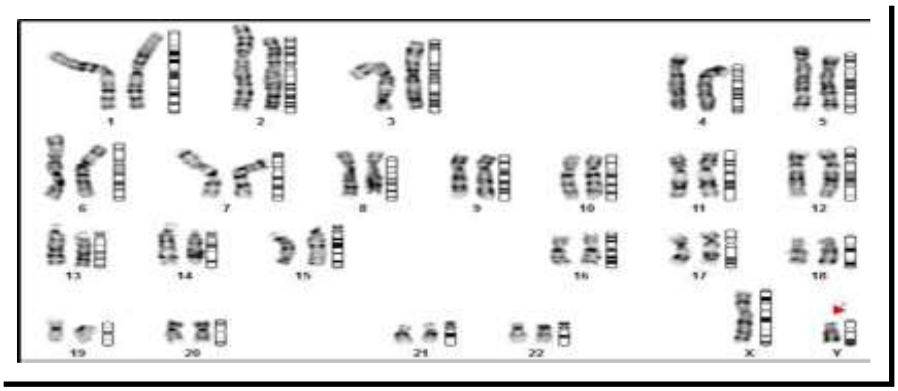

Figure 2: 46, XY DSD –Abnormal with sex reversal chromosome

14. Gonadal dysgenesis

Gonadal dysgenesis seems to be a term used to describe a set of diseases that affect the development of the gonads, which include the testes and ovaries [68]. Turner syndrome is the most well-known of these diseases, affecting one out of each 2500 female live births as well as causing a slew of manifestations and consequences [69]. Gonadal dysgenesis develops during the embryonic and fetal phases, either during fertilization or shortly after. 46, XX or 46, XY are the genotypes for complete gonadal dysgenesis. Genetic abnormalities or environmental factors impacting ovarian development might cause the 46, XX type, which results in ineffectual ovaries. In the scope of disorders which promote primary ovarian insufficiency, gonadal dysgenesis (46 XX) is one of them. This Swyer syndrome commonly known as XYvariety of CGD (complete gonadal dysgenesis), it is caused by gene mutations in SRY gene is found on the Y-chromosome sex-determining region; over here numerous feasible genes were involved, although the actual cause in many cases remains unknown [68]. Turner syndrome individuals were found to exhibit several genotypes. Partial gonadal dysgenesis is defined as the chromosomal anomaly (45, XO) and 45, X/46, XY mosaicism or X0/XY mosaicism , 45X/ 47, XXX/46, XX karyotype (Figure 3). Isochromosome X Long arm, Xq (46, X,i) and ring chromosome (46,X,rX) deletions are also develope in certain mosaics. Mosaic variants of the disease, as expected, have less symptoms and phenotypic manifestations due to the presence of normal genetic information in some cell lines. The manifestations as well as the phenotypic appearance of mosaic types of the disease are less severe, as a result of certain cell lines possessing proper genomic information

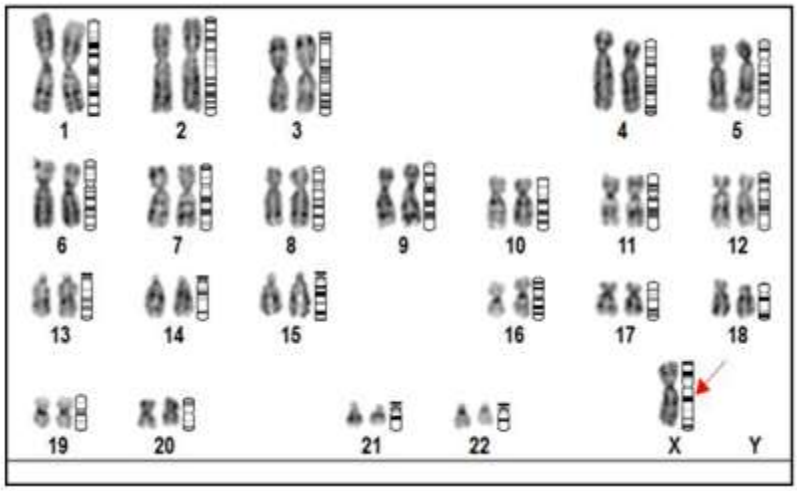

Figure 3: 45, X – Turner Syndrome

14.1. 46, XX, Pure Gonadal Dysgenesis

Gene mutations in 21-hydroxylase are the most common cause of pure gonadal dysgenesis (46 XX type) associated with adrenogenital syndrome/congenital adrenal hyperplasia.The hydroxylation process of 11 deoxycortisol from 17-hydroxyprogesterone is usually carried out by 21-hydroxylase enzymes. When this enzyme is weak, it reduces cortisol synthesis, which raises ACTH, which enhances cholesterol consumption in cells promote the synthesis of Pregnenolone. High level of precursor of steroid cause virilization by shunting testosterone synthesis [70]. Gene mutations encoding the ovary's FSH receptor

also linked to 46XX gonadal dysgenesis in some cases. Due to G protein-coupled receptor mutation, signalling is reduced/absent, resulting in diminished ovarian follicle development. BMP15, a gene that encodes the TGFB-transforming growth factor beta protein implicated on ovarian folliculogenesis, is also engaged with 46 XX gonadal dysgenesis. These gene's derivatives also promote granulosa cell growth. Transforming growth factor-beta proteins are initially converted to single-peptide precursor proteins containing both pro and mature region. For effective processing of post translational modification to make functionally active proteins, different elements of pre-protein are essential.The absence of such active proteins are thought may be linked to diminished follicle development and also ovarian follicle reduction [71].

Complete gonadal dysgenesis (46 XX CGD) is congenital within recessive autosomal form, and various genes locus have been involved; unfortunately, the actual mechanisms underlying this form of gonadal dysgenesis remain unknown. Deactivated follicle-stimulating hormone receptor (FSHR) gene mutation have been associated with gonadal dysgenesis primarily among the Finnish people. Follicular development is halted when this receptor is mutated, causing follicles to persist in the primary phase and the second phase of follicle development/antral stage. This mutation also causes the follicles to be depleted [71]. Furthermore, researchers have looked for other genes that may be linked to gonadal dysgenesis (46XX) and BMP15 gene appears to become a likely possibility. The definitive relationship to gonadal dysgenesis, however, has yet to be established.Gene BMP15 which encodes for TGF beta proteins other than those produced by oocytes. In several investigations, an unusual amino acid replacement in BMP15 gene at p.A180T was found in a limited percentage of patients suffering from ovarian failure, according to future investigations, these amino acid alteration may indicate an uncommon polymorphis [71].

14.2. Pure 46XY, gonadal dysgenesis (PGD)

SRY gene mutations are considered to be responsible for 15% over all instances of 46 XY PGD. Mutations have been frequently discovered within the DNA binding region's high mobility group.In the testes, an SRY gene highly encoded in the initial Sertoli cells that develop and contributes to the integration the series of events that led to sexual difference.Sertoli cells are responsible Leydig cells grow, it can subsequentlyrelease androgens and insulin-like factor3, causing Mullerian duct structures to regress and Wolffian duct structures to differentiate [70]. When SRY is mutated, the result is a complete gonadal dysgenesis/Swyer syndrome or underdeveloped gonads /streak gonad, whereas when SRY is healthy, certain premature seminiferous tubules can be seen as well as similar genes influence dysgenesis [72]. Based on the research findings of chromosomal aberration, and variant pathogenicity the genes that encode such factors in Disorders of Sex Development (DSD) patients, Sex determination is influenced by a number of transcription factors and signalling molecules, the role of signalling molecules on sex determination has been discovered [70]. The MAP3K1 mutations were found to have gained of functional consequences, such as phosphorylation targets and reduced SOX9 expression. SOX9 is required for testicular development and has been connected to -catenin, another sexual development component [73].

The Y chromosomes SRY gene activates testicular development pathways by acting as a switch. The other important gene essential in the development of the male reproductive

organ is SOX9 that is associated with the effect of inhibitory material for Mullerian Mullerian. The Mullerian inhibitory substance is responsible for preventing the development of female internal genitalia, allowing male development to proceed. Furthermore, MAP3K1 is a prevalent gene associated with (46, XY) complete gonadal dysgenesis, with 13 to 18% of those affected. MAP3K1 MAP3K1 downregulate the expression of SOX9, causing signalling to resemble ovarian development, leading to abnormal testicular development. Causing signalling to resemble that ovarian development, resulting in aberrant testicular development. Gonadal development is hampered when the testicular development pathway is downregulated [73].

15.Enzyme Defeciencies

15.1. 5-Alpha-reductase Deficiency

In a 46-year-old XY woman, a 5-Alpha-reductase deficiency can cause primary amenorrhea. Due to an inability to metabolize testosterone to its more potent metabolite dihydrotestosterone, these patients may have ambiguous genitalia at birth (DHT). Two-thirds of 5-RD2 patients who were initially given a female gender live as men, whereas all patients who were initially assigned male gender live as males [19]. They can become virilized during puberty due to the typical peripubertal rise in testosterone release without developing breasts. These people, on the other hand, do not have DHT-dependent expansion of the male external genitalia. Multiple factors influence management options, including reproductive anatomy, DSD aetiology, parental/cultural influences, and, most significantly, outcome [74].

16. Hyperprolactinemia

Hyperprolactinemia has numerous effects on the reproductive endocrine axis. The most prevalent causes of hyperprolactinemia are prolactinoma and drug-induced hyperprolactinemia (DIH). Because medicines and the pituitary tumour itself can alter the reproductive endocrine axis, menstrual cyclicity may be affected differently in people with DIH and prolactinoma [75]. Antipsychotic medicines, for example, modify other neurotransmitters, including histamine, gamma-aminobutyric acid (GABA), and opiates, which might impact the hypothalamic-gonadal axis and cause hyperprolactinemia. Patients with prolactinoma, on the other hand, may experience gonadotrope malfunction as a result of the tumor's bulk effect [76]. Menstrual cyclicity may also be affected by related thyroid hormone and corticotroph insufficiency. Menstrual cyclicity and responsiveness to medication may differ in patients with prolactinoma and DIH due to the distinct etiologic profiles.

13. Conclusion

Amenorrhea is a menstrual-related issue that affects women worldwide. As a consequence, it is critical to determine the variables that cause primary/secondary amenorrhea in adolescent girls and women in order to ensure their reproductive health. As per our study, most Amenorrhea patients are identified with Turner syndrome and secondly with swyer syndrome. Chromosomal abnormalities in amenorrhea patients were identified by Karyotyping, FISH analysis and Microarray analysis.

Declaration of Competing Interest

There is no conflict of interest among the authors.

Acknowledgments

I would like to thank Mr. Mayur Abaya, Managing Director of Life Cell International Pvt. Ltd for giving opportunity and permitting to carry out the study. I also thank all technical staff of the cytogenetic department.

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