CHAPTER II

LITERATURE REVIEW

2.1. Development of the Reproductive Systems

The basic processes of normal development are sex determination and sex differentiation, development of the hypothalamic-gonadotrophic axis in fetus, and function of the hypothalamic-pituitary-gonadal (HPG) axis in childhood.[16]

2.1.1. Sex Determination and Sex Differentiation

Sex determination is the process whereby the bipotential gonad develops into a testis or an ovary. Sex differentiation requires the developing gonad to function appropriately to produce peptide hormones and steroids. Classically, sex determination and sex differentiation can be divided into three major components chromosomal sex (i.e., presence of a Y or X chromosome), gonadal sex (i.e., presence of a testis or ovary), and phenotypic or anatomic sex (i.e., presence of a male or female external and internal genitalia).

2.1.1.1. Chromosomal Sex Determination and Differentiation

The chromosomal sex determination is the first step of sex differentiation in human. An oocyte has a chromosome complement of 23, X and a sperm has a complement of either 23, X or 23, Y.[37] Chromosomal sex usually is determined at the time of fertilization, when two haploid gametes (ova and sperm, with 23 chromosomes each fuse to generate a diploid zygote (46 chromosomes). Gametes are ultimately derived from germ cells, which initially replicate their chromosome complement and then undergo a series of two meiotic divisions, meiosis I (reduction division) andmeiosis II, to produce haploid ova or sperm. This fusion

resulting, respectively, in a 46, XY (genetic male)or 46, XX (genetic female) zygote after fertilization.[16]

2.2. Definition and Prevalence

Amenorrhea is an absence of menses in female of a reproductive age, the first menstrual cycle (menarche) is the last feature of maturity in female.[3] Amenorrhea is divided to a primary and secondary, primary amenorrhea means failure of menarche, associated with no development of secondary sexual signs by age of 13 years, or failure of menarche with well-developed secondary sexual signs by the age of 16 years.[1] Secondary amenorrhea means cessation of menstrual cycle after reaching normal menarche. The incidence of primary menorrhea worldwide estimated to be 1%,[4,5] and from the research results of different countries, there was no evidence for higher frequency in specific population or ethnic group.[2,4]

2.3. Pathophysiology

The basic understand of menstrual cycle and the hormonal changes are the key of evaluation and management of its disorders.[6] For the regular and normal menstrual cycle the basic needs are intact endocrinal axis (hypothalamus-hypophyseal, ovaries). intact uterus with competent endometrium that can respond to hormones, and normal external genitalia is important.[1] The hypothalamus produce the gonadotropin releasing hormone (GnRH) then it transfer to anterior pituitary and stimulate the gonadotrophocytes, in response to this stimulation these cells secretes the follicular stimulating hormone (FSH), and sex hormones (Estrogen, Progesterone and Testosterone). Figure 1 show the physiology of menstrual cycle.

2.4. Etiology of primary amenorrhea

Establishing the etiology of primary amenorrhea is important for effective management, the causes of primary amenorrhea is divided according to the source of the disorder.

2.4.1. Disorder of the anatomical aspect of reproductive tract

2.4.1.1. Mullerian Anomalies

Any patients presenting with primary amenorrhea the mullerian anomaly should be ruled out. [17] Hence, imperforate hymen, obliteration of vagina or lapses in continuity of the vaginal canal should be ruled out by examination. There is some patients with complete absence of the uterus or cervix.[11,14,17]

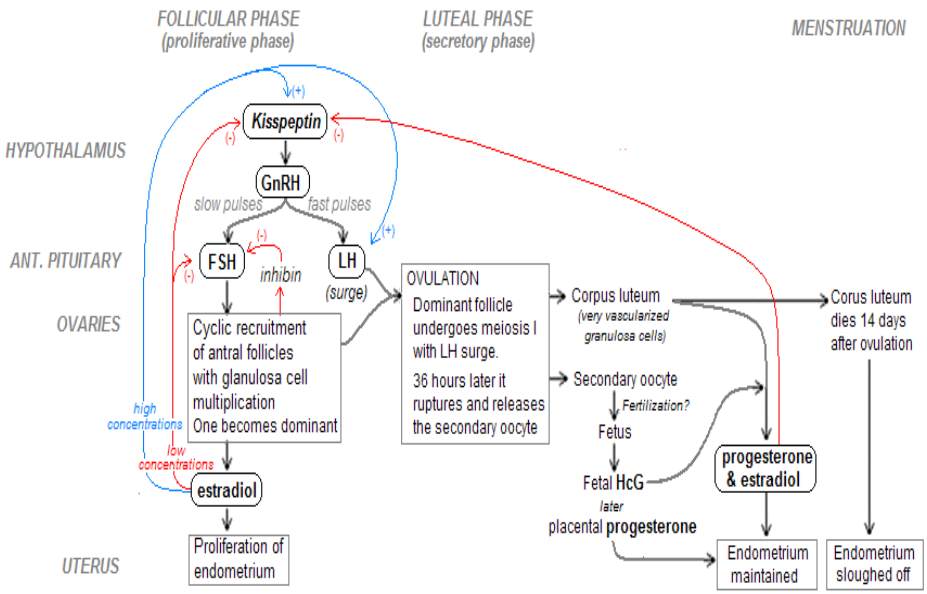

Figure 1. Physiology of Menstrual Cycle

The hypothalamus, anterior pituitary, ovaries and uterus are the four structures responsible for normal menstrual cycle. Hormones released in the hypothalamo-pituitary –ovarian (HPO) axes are regulated

by a negative feedback mechanism on the gonadotrophocytes in the anterior pituitary and indirect inhibition at the hypothalamic level. *Best Pract Res Clin Endocrinol Metab*. 2010;24(2):163–86.) [45]

Less common patients present with a uterus with no cavity or the uterus is present, but the cavity is absent or the presence of a cavity with congenital lacking of the endometrium. With the later exception such patients presents with amenorrea in addition to a haematocolpos, haematometra, or haemoperitoneum.[25]

2.4.1.1.1. Mullerian Agenesis-Mayer Rokitansky-Kuster-Hauser Syndrome (MRKH)

It accounts 15% of primary amenorrhea patients and a prevalence of 1:5000 female births.[11] It is the most common after Turner syndrome. Affected individuals have a 46,XX karyotyping and a normal secondary sex characteristics. Type A (MRKA) patients show Symmetric uterine buds and fallopian tubes. Type B (MRKH) shows asymmetric uterine buds and fallopian tubes and being associated with other congenital anomalies (skeletal, renal, ovarian, ear and cardiac).[14]

Patients with MRKH syndrome have an absence or a hypoplasia of the vagina, with usually absence of uterus and fallopian tubes.[8] Rarely, the uterus may be normal but lacking a passage to the introitus, or there maybe just rudimentary, bicornuate cords present.[11]In patient of partial endometrial cavity, cyclical abdominal pain may be a complaint. It is important to get a female karyotype because cof similarities of some male pseudohypogonadism.[17] Causes 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.[18]The WNT gene family consists of structurally related genes which encode secreted signaling proteins. These proteins have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis.[41] This gene is a member of the WNT gene family, and is the first signaling molecule shown to influence the sex-determination cascade.[42] It encodes a protein which shows 98% amino acid identity to the Wnt4 protein of mouse and rat. This gene and a nuclear receptor known to antagonize the testis-determining factor play a concerted role in both the control of female development and the prevention of testes formation.[44] This gene and another two family members, WNT2 and WNT7B, may be associated with abnormal proliferation in breast tissue, mutations in this gene can result in Rokitansky-Kuster-Hauser syndrome.[40]

2.4.1.1.2. Imperforate Hymen

Patient with imperforated hymen presents with cyclic pain and primary amenorrhea.[19] Physical examination often reveals a bulging hymen with a bluish hue. Some patients also present with dysuria, back pain, constipation and painful defecation, as well as primary amenorrhea.[13] This condition must be differentiated from a low transverse vaginal septum.

2.4.1.1.3. Transverse vaginal septum

Is a rare anomaly of the female genital tract, the incidence has been reported to be 2 in 100, 000 female live births.[20] The etiology of transverse vaginal septum is unknown, although most patients are thought to be the result of female sex-limited autosomal recessive transmission. In transverse vaginal septum a vertical fusion disorder exists between the Müllerian ducts and the urogenital sinus.[19,20] The septa may occur at any level in the vagina with the following frequencies: 46%, upper vagina 40%, mid vagina14%, lower vagina. Septa

can be complete or incomplete. They are generally less than 1 cm in thickness, with thicker septa noted to be more common near the cervix.[36]

2.4.2. Disorders of the gonads

More than half of primary amenorrhea patients are due to abnormal gonadal differentiation or abnormal function during the early fetal and neonatal development. About 40% of primary amenorrhea patients the cause is gonadal dysgenesis in phenotypic female who are genotype male 46,XY. [37]

Gonadal dysgenesis relatively rare conditions most often present in the newborn or the adolescent periods. In the newborn, patients usually come with atypical genitalia and affected adolescent patients come with considering atypical sexual development during pubertal age.[21]

2.4.2.1. Gonadal Dysgenesis (Turner Syndrome)

Turner syndrome is the most common chromosomal anomaly associated with primary amenorrhea.[6] Turner syndrome occurs in 1/2, 500 to 3, 000 live female births.[22] The syndrome is characterized by the partial or complete absence of one X chromosome 45,X (classical Turner syndrome); 45,X\46,XX(Turner variants) 45, X\46, XY(mixed gonadal dysgenesis) is often found in those patients.[8] About 13.5-15% of Turner syndrome have a duplication (Isochromosome) in the long arm of chromosome X [46, X, i(Xq)], Ring -46, X, r(X), deletions-46, X, del(Xp).[5]

Chromosomal anomaly (monosomy X) happens randomly since the process of fertilization because; non 1`1`X` 1disjunction of X chromosome when meiosis happen from the maternal side. Mosaic Turner also not inherited, there was randomly during the cell division at the beginning of the development of the embryo.[23]

The clinical features of the syndrome including short stature (< 150 cm), short fourth and fifth metacarpal bones, webbed neck, wide spaced nipple, high arched palate, low posterior hair line, low setted ears, cubitus valgus, cardiovascular and renal anomaly. Females with Turner syndrome typically have normal intelligence.[2]

Other differential diagnosis with Turner syndrome and has the similar clinical features is Noonan Syndrome. Clinicaly the patients had short stature, short neck, webbed neck, low posterior hair line, shield chest and wid intermammary distance.[24] The characteristic of chest of those patients usually have pectus excavatum in upper chest and pectus carinatum in lower chest, those patients do not have gonadal dysgenesis,[25] so female with Noonan syndrome can menstruated normally. Cardiovascular anomaly seen in 80% of NS patients.

Screening of all patients with gonadal dysgenesis is important Because; the possibility of component Y chromosome and risk of carcinogenesis.[9] Using molecular techniques as FISH can identify the structure of the Y chromosome other than examination of immunological to the H-Y antigen help to know the risk of tumor.[14] Women with these circumstances have a high level of FSH in the absence of ovarian follicles and reduction in the negative feedback mechanisms on the FSH of estradiol and inhibin.[5]

2.4.2.2. Pure Gonadal Dysgenesis

One of the causes of the hypogonadism in the females is 46, XX gonadal dysgenesisis, which leads to nonfunctioning gonads,[26] despite of the normal genotype female gonadal dysgenesis can be sporadic or familial. The mode of inheritance is autosomal recessive, and the mutation located on the short arm of chromosome 2,[21] there are gene mutation in the FSH receptor. Sporadic pure gonadal dysgenesis associated with trisomy 13 and 18 because the possibility of the existence of ovarian gene on the chromosome 13 and 18.[14] Phenotype in females with pure gonadal dysgenesis including: normal stature, sexual

infantilism, bilateral streak gonads, primary amenorrhea and high plasma level of FSH and LH.[8]

2.4.2.3. 46,XY gonadal dysgenesis (Swyers syndrome)

The presentation of this patient is a phenotype of normal female with normal internal and external genital, as well as streak ovaries.[9] In spite of male karyotype, the gene responsible for this disorder is sex reversing locus (SRVX) in the short arm of X chromosome (Xp21.2-p22.11) responsible for the patient of XY reversal with karyotype 46,XY.[26] The presence of Y chromosome has a risk of malignant changes.[4] So, prophylactic gonadectomy should be done as soon as the diagnosis confirmed.

2.4.2.4. Mixed gonadal dysgenesis

This is a rare anomaly of a symmetrical gonadal development, with a germ cell tumor or testis and streak or no ovaries.[4] The condition can be classified as a Turner variant (45, X \46, XY), or (46,XX/46,XY) those patients have ambiguous external genitalia and exhibit virilization after puberty.

2.4.2.5. Primary Ovarian Failure (POI)

Primary ovarian failure is a condition characterized by hypoestrogenemia, elevated serum level of gonadotropin associated with amenorrhea.[14] In women with primary amenorrhea the prevalence of POI accounts 10%-28% of the causes genetic disorder associated with POI including Turner syndrome, Klinfelter syndrome, mosaicism and specific structural abnormalities on sex chromosomes.[28] searching for 45,X/46,XX mosaicsm using FISH a larger percentage of cell containing single X can be detected in women who present with premature ovarian failure.[27] Translocations in the critical region on the long arm of X

chromosome is also mechanism of ovarian failure is mostly due to accelerated follicular atresia as in Turner syndrome.[14]

2.4.3.Disorders of Hormonal and Receptor Target Organs

2.4.3.1. Complete Androgen Insensitivity Syndrome (CAIS)

The patients present with blind vaginal canal and absent uterus. This is the third mostcommon cause of primary amenorrhea, aftergonadal dysgenesis and mullerianagenesis.[14,27] The patient with CAIS is a male pseudohermaphrodite. Hence, the other name of the syndrome is testicular feminization. The adjective sex refers to the gonadal sex; thus the individual is having a testis and a male karyotype. Phenotypically the CAIS is female but with absent pubic and axillary hair. Mode of inheritance in CAIS is X Linked recessive.[28]

The diagnosis should be suspected in i) inguinal hernias in female child, because the testis are generally partially descended. ii) a patient with primary amenorrhea and absent uterus iii) a patient with absent body hair.[14] In this syndrome a combination of normal female phenotype, male karyotype and normal or high blood testosterone levels along with high luteinizing hormone (LH) level is present.[29]

The physical criteria including large breast with small nipples and pale areolae.[12,14] More than half of patients 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. Intraabdominal testis, there is high incidence of neoplasia in these gonads. The incidence of malignancy 22% and of neoplasia 52%.[14]

Therefore once puberty attained, gonadectomy should be done. and the patient should start hormonal therapy. Which is the only patient where gonads with a Y chromosome should be removed as soon as diagnosis is made. Because; the development achieved with hormone

treatment does not match the smooth pubertal changes due to endogenous hormones and gonadal tumors have not been encountered before puberty in these patients.[28] Checking 17ketosteroids were found to be normal. Plasma Testosterone are in the normal to high normal range, along with normal metabolism and clearance. Hence having normal testosterone levels these patients do not respond either to endogenous or exogenously given androgens. Because the critical step required for sexual differentiation is androgens response so, the development is totally female.[39] Since AMH is present development of mullerian structures completely gets inhibited, leading to absence of uterus phalobian tubes and an upper vagina.[27]

Partial androgen insensitivity syndrome occurs in 1/10th of those number of androgen insensitivity (AIS) patients,[27] the clinical features is range from normal female phenotype with clitoromegaly and fusion of labia majora to normal male phenotype with micropenis, hypospadias and unilateral or bilateral cryptorchidism.[30] Where individuals have some androgen effect. They may have a mild clitoromegaly or have a phallus developed.[14] The axillary and pubic hair developed along with a breast. Gonadectomy should be done early to prevent further undesirable virilization.[12,28,30]

2.4.3.2. Ovarian Insensitivity Syndrome (Savage Syndrome)

Those patients have defect in the function of the ovaries,[1] it expected to be due to defect in the receptors of the gonadotropin cell membrane.[40] So, the ovary not respond to hormonal stimuli. The patients also have a high plasma level of FSH and LH.[31] For most patients presenting with POI, the cause is largely unexplained. Potential etiologies for POI can be divided into genetic, autoimmune, metabolic dysfunction, infectious, and iatrogenic categories.[40]

2.4.4. Disorder of Hypothalamus

The hypothalamus is the source of GnRH, which is directly stimulates gonadotropic pituitary synthesis and secretion.[32] Dysfunction at this level leads to hypogonadotropic hypogonadism or eugonadotropic hypogonadism. Hypothalamic dysfunction results in decreased GnRH secretion,[8] affect pulsatile release of LH and FSH resulting in an ovulation.[32] The patients came with amenorrhea, undeveloped secondary sexual signs. Because; low estradiol, with normal height and normal body hair distribution.[32]

2.4.4.1. Hypothalamic Idiopathic HypogonadotropicHypogonadism (IHH)

Patients with IHH presenting with delayed or absent pubertal development, due to a Gonadotropin releasing hormone (GnRH) mutation or a GnRH deficiency. Usually it affects males, but can present in women as a cause of primary amenorrhea. The most common phenotypic association is anosmia. Kallmann's syndrome is a very rare cause of primary amenorrhea.[33] It is characterized by hypogonadotropic hypogonadism associated with anosmia or hyposmia, both of which occur as a result of impairment of olfactory axon development and failure of migration of gonadotropin-releasing hormone (GnRH) neurons.[7]

Mode of inheritance can be autosomal dominant, autosomal recessive, or X-linked.Because; shared embryonic origin of GnRH neurons and olfactory neurons.[34] The causes of IHH can be due to mutation in KAL-1 gene, which located on the pseudoautosomal region of the short arm of X chromosome. Associated with facial midline defect and neurological deficiency.[14] Mode of inheritance of the syndrome is X-linked recessive disorder. In females the specific mutation in Kal-1 gene with hypogonadotropic hypogonadism has not been identified.[34]

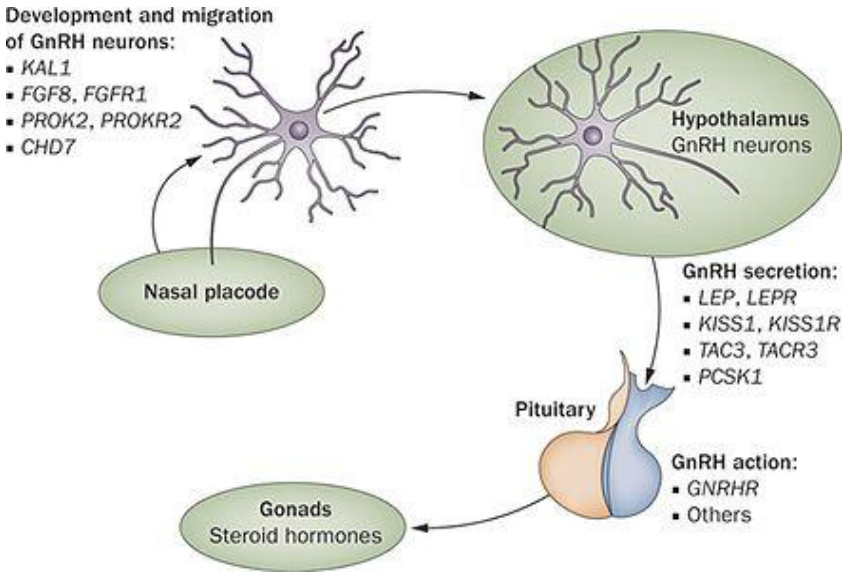

Figure 2.Development and Migration of GnRH

Most GnRH neurons orginate from stem cells in the nasal placode and migrate to the hypothalamus. The strongest activator of the GnRH neurons is kisspeptin hormone. The genes that are responsible for development and migration of GnRH neurons including KAL1,FGFB,FGFR2,CHD7.((Taken from Williams textbook of Endocrinology, 12th Ed, Chapter 23, Page 881) [46]

2.4.5. Disorder of pituitary

Some mutations affecting the pituitary can cause amenorrhea, the clinical manifestation is delayed puberty and infertility.[35] In patient of females with low FSH and LH the possibility is mutation in the receptor of GnRH,[32] other genetic abnormalities associated with primary amenorrhea is mutation in the FSH gene.[14] The mode of inheritance is autosomal recessive. Causing low serum level of FSH and estradiol with clinical features of delayed puberty and primary amenorrhea.[36] Twenty seven mutations in the PROP-1, transcription factor pituitary, this lead to low level of TSH, PRL and GH.[14] Those patients present with short stature, hypothyroidism, delayed puberty and primary amenorrhea.[34] primary amenorrhea due to hyperprolactinemia associated with suppression of GnRH of the

hypothalamus and inhibition of LH and FSH, suppress the gonadal function as well as galactorrhea.[6]

2.4.6. Disorder of Adrenal Gland

2.4.6.1. Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is a disorder of the adrenal cortex characterized by deficiency of corticosteroid, with or without aldosterone deficiency, associated with excessive circulating androgen.[37] The clinical phenotype is typically classified as classic, the severe form, or non-classic. The congenital adrenal hyperplasia is rare cause of primary amenorrhea. The clinical picture of the patient include hairsuitism, ambiguas genitalia.[37]

2.4.6.2. Adrenal Hypoplasia Congenital (AHC)

It is an X linked disorder caused by mutations in DAX-1 gene. AHC is characterized by adrenal insufficiency and delayed puberty due to hypo gonadotropic hypogonadism. The patients usually a males genotype but they have a females phenotype. very rarely the disorder can be caused by steroidogenic factor 1 SF1.[42]

2.6. Cytogenetic analysis in Primary Amenorrhea

From the literature review it has been reported that the percentage of chromosomal abnormalities varies from 15.9%to 63.3%.[3] Several cytogenetic studies were carried out to understand primary amenorrhea but most patients were selected for karyotyping on the basis of certain severe clinical features and not all of them were screened for karyotypic analysis.[41] Hence the frequency of chromosomal abnormalities associated with primary amenorrhea has to be considered with certain reservation. PA in the majority of patients caused by sex

chromosome anomalies it involve numerical variations and structural rearrangements. Aneuploidy results in XO or XXX conditions observed include isochromosomes, partial deletions, X-autosome and X-X translocaions and rings, in addition to other types of anomalies.[6]

Evidences have mounted in literature to show that a number of sex chromosomal abnormalities in women are associated with PA.[1,8,35] Wide ranges (15.9-54.7%) of frequency of chromosomal anomalies are associated with PA.[8] These reported variations in the frequency of total chromosome anomalies may be due to the variation in the methods of ascertainment of patients, selected for karyotyping.[8]

2.7 Clinical features in primary amenorrhea

2.7.1 Height and weight in primary amenorrhea

The menarche age is often considered for various reasons. It is one of the major indices of the female fertility.[4,38] There have been studies on the role of height, weight, and body structure on the menarche age; however, there is a variation on the role of such factors. Some researchers believe that some body fat is necessary in female adolescents and there is a minimum weight requirement for starting the menstruation. Higher food consumption which is often a result of the improved socioeconomic status is among factors leading to the lowered age of menstruation, as witnessed in the present century. [39]

Short stature is the most frequent characteristic in Turner syndrome patients. The mean height for adult female with Turner syndrome is 140cm, the cause of short stature in Turner syndrome suggested to be that the homeobox gene, *SHOX*, in the pseudoautosomal region is the major player and that haploinsuffiency of this gene leads to the growth failure.[38] And it may be the responsible for the increased stature seen in other sex chromosome aneuploidy conditions such as 47,XXX, 47,XYY, 47,XXY, 48,XXYY.

The other possible cause of short stature in Turner syndrome is inadequate production of Estrogen, many of those with Turner syndrome develop osteoporosis. This can decrease height further, as well as exacerbate the curvature of the spine.[14]

2.7.2 Tanner stage

The Tanner stage is the measurement of secondary sexual sign including the breast development and pubic hair. It consist of 5 stages, the stage 1 is elevation of papilla only and no pubic hair, stage 2 is breast bud: elevation of breast and papilla as a small mound and enlargement of areola diameter and the pubic hair is spare slightly pigmented downy hair along the labia. Stage 3 is additional enlargement of breast and areola with no separation of their contours the pubic hair is spreads sparely over the pubic region and is darker, coarse and curlier. In stage 4 the areola and papilla project from the surface of breast to form secondary mound. Stage 5 is mature stage with projection of papilla only wit ׀recession of the areola to the general contour of the breast pubic hair is adult in quantity and type. Figure 3 show the different stages of Tanner staging.

Axillary hair is a type of puberity scaling and goes through 4 stages of development, driven by weak androgens produced by the adrenal in males and females during adrenarche, and testosterone from the testicle in males during puberty.[42]

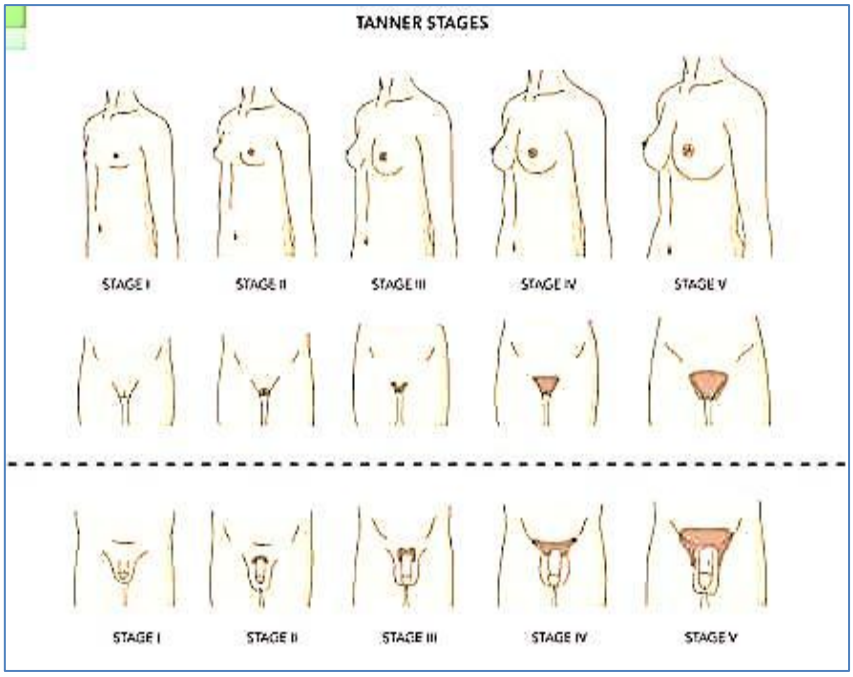

Figure 3. Tanner staging of breast and pubic hair

Tanner stage is the measurement of secondary sexual sign including the breast development and pubic hair consisting of 5 stages
The photo taken from (*emedicine.medscape.com*.) [47]

Like Tanner Staging for pubic hair, axillary hair can be staged according to the staging system, named for Pediatric Endocrinologist, Dr. Joseph Wolfsdorf, as follows:

1. Wolfsdorf Stage 1- no axillary hair
2. Wolfsdorf Stage 2- scant axillary hair (usually coinciding with onset of adrenarche)
3. Wolfsdorf Stage 3- coarse axillary hair, less than full-adult
4. Wolfsdorf Stage 4- full adult axillary hair

2.7.3 Prader stage

The Prader scale or Prader staging is a coarse rating system for the measurement of the degree of virilization of the genitalia of the female and is similar to the Quigley scale. It primarily relates to virilization of the female genitalia in patients of congenital adrenal

hyperplasia (CAH) and identifies five distinct stages, but in recent times has been used to describe the range of differentiation of genitalia.

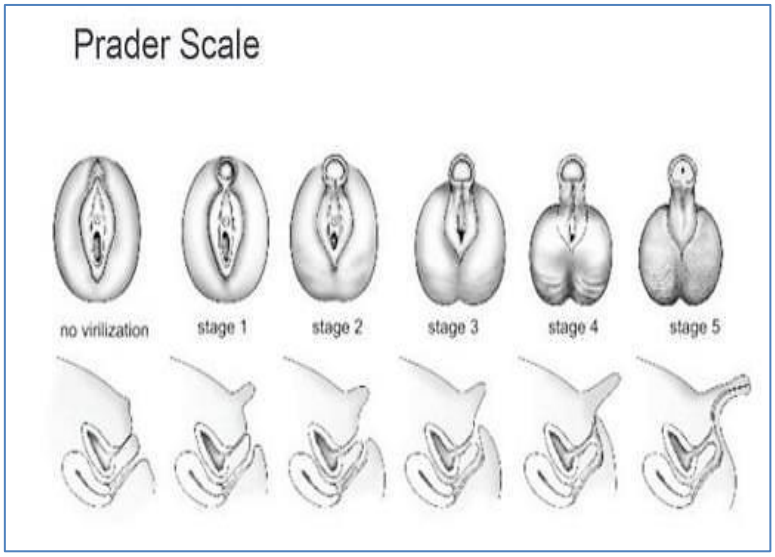

Figure 4. Prader staging

Prader stage is a meaureing for masculinization of female genitalia and consisting of 6 stages start from 0 as normal female to stage 5 which is a virilized female**.** The photo citied from **(** *Best practice and research. Clinicalendocrinology and metabolism)* [45]