زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

Prevalence of hyperprolactinemia In women

Research Project
Submitted to the department of (Chemistry) in partial fulfillment of the requirements for the degree of B.Sc. in (Chemistry)

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

وما اوتيتم من العلم
الا قليلا
صدق الله العظيم

CERTIFICATE:

This project has been written under my supervision and has been submitted for the award of the degree B.Sc. in chemistry with my approval as supervisor

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Signature

Date: / / 2024

I confirm that all requirements have been fulfilled

Research project lecturer

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Signature:

Date : /4/2024

Dedication

To all my family members and friends**.**

To all my teachers, especially my supervisor.

To all who helped me to learn .

Acknowledgment

I'd like to thank the Lord of the Universe for everything (ALLAH). Also, I want to express my heartfelt gratitude to the Kurdistan regional government, the Salahaddin university presidency, and the higher education council. I'm grateful to the deanery of the college of education – chemistry department for everything that helped me, especially **(Dr. Dler Dlshad Kurda).** Special thank is due to my supervisor **Dr. Parwin Abdulsamad** helped me, with stimulating suggestions and encouraged me at all times of the fabrication process and in writing this report. I also sincerely thank you for the time spent proofreading and correcting my many mistakes.

Rudaw ismahil mulud

Abstract

This study deals with hyperprolactinemia, its causes, associated symptoms, and current treatment. The objective is to review recent articles concerning pathophysiology, treatment, and therapy resistance. Hyperprolactinemia is a relatively common endocrine abnormality caused by an in- creased secretion of prolactin (PRL) from the pituitary gland. Hyperprolactinemia causes an ovulation and infertility but can affect women of all ages and therefore should be considered in issues dealing with reproduction and menopause. The etiology as well as the natural history of hyperprolactinemia can vary widely. Better awareness in this regard is important in order to determine treatment and evaluation of patients with hyperprolactinemia .

List of abbreviation

PRL = prolactin

T3 =triiodothyronine

T4 = thyroxine

FSH =follicle stimulating hormone

LH =luteinizing hormone

PRLR =prolactin receptor

ACTH =adrenocorticotropic hormone

GH =growth hormone

TSH=thyroid stimulating hormone

OT =oxytocin

ADH=antidiuretic hormone

TRH =thyrotropin-releasing hormone

GnRH =gonadotropin-releasing hormone

% = percentage

ng= nanograms

mL =milliliter

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CHAPTER ONE

1.1Introduction and Literature Review

1.Introduction

Prolactin (PRL) is a polypeptide hormone of a pituitary origin, whose production is controlled by dopamine .This prolactin hormone has many biological activities such as lactation and reproductive functions (Bernichtein et al., 2010)It is produced mainly by the anterior pituitary gland and also produced locally by multiple extra pituitary sites where it acts in an autocrine/paracrine manner(Terasaki et al., 2010). Hyperprolactinemia is a condition of the presence of abnormally high levels of prolactin in the blood in which the normal levels are 10-21 mIU/ml. This condition is present as a pathological condition (Davis, 2004)An excessive prolactin secretion decreases the level of gonadotropin releasing hormone impairing the pituitary production of follicle stimulating hormone and luteinizing hormone. It also impairs directly the endocrine activity of ovarian follicles so it will affect the ovulation (Kubba, 2016)Thus hyperprolactinemia represents a common problem in the reproductive dysfunction, in which it leads to high circulating levels of prolactin and hypogonadism which lead to lack of gonadotrophin cyclicity and to infertility. The clinical feature can range from irregular cycle, oligomenorrhea, amenorrhoea and galactorrhoea. Mild hyperprolactinemia can cause infertility even when there is no abnormality in the menstrual cycle (AbdElghani and Elmugadam, 2013)The human prolactin gene is present as a single copy on chromosome 6 it is about (12.215 kb). It contains 5 exons and 4 introns and the transcription of it is regulated by two promoters. Upstream, it is used in extra pituitary cells and tissues and down stream promoter that directs the transcription in pituitary lactotrophs (Rui, 2000)The effects of prolactin are mediated by the interaction with its receptor (PRLR). The binding of prolactin activates the pre-dimerized prolactin receptor and results in the activation of prolactin receptor-associated signaling cascades such as Jak2/Stat5 resulting in the transactivation of prolactin-responsive genes (Fang et al., 2010)As the prolactin is an essential regulator of mammary development. The primary cells targeted by prolactin are the breast tissue cells which are involved in the development of mammary gland and in cellular growth and differentiation as well as in the initiation and maintenance of lactation (Courtillot et al., 2010).

1.2. Literature Review.

1.2.1 The Endocrine system.

The endocrine system is an integrated system which consists of several glands, hormones and scattered hormone secreted cells (Kester et al., 2004)Al-though vertebrate endocrine systems vary, they consist of the same basic glands and hormones. This system helps maintain homeostasis, integrate and coordinate many diverse physiological functions. It coordinates with the nervous system in other vital communication functions within the animal's body .These two systems work together to produce a variety of responses from sexual and reproductive behavior to control the growth and development and to adjust the delicate chemical balance of body fluids (Kubba, 2016). The major endocrine glands include the pineal gland, pituitary gland, parathyroid gland, hypothalamus and adrenal glands. These glands produce different types of hormones that evoke a specific response in other cells, tissues and/or organs located throughout the body. Many endocrine glands discrete organs whose primary functions are the production and secretion of hormones (Le et al., 2013). Hormones are of different classes based on their chemical composition and they can be divided into chemical classes such as amines which are derived from the amino acids tyrosine and tryptophan such as hormones derived from the thyroid glands, such as triiodothyronine (T3) and tetraiodothyronine (thyroxine, T4) which make up a subset of this class because they derive from the combination of two iodinated tyrosine amino acid residues. Polypeptide and proteins in which polypeptides such as antidiuretic hormones. Proteins are polypeptides with more than 100 amino acids like growth hormone. Glycoproteins consist of a long polypeptides . All hormones secreted by the pituitary gland are peptide hormones, such as FSH and LH. Steroid hormones are lipids derived from cholesterol such as the leptin.

1.2.2 Pituitary gland.

This gland is under the control of the hypothalamus which decides how the hormones are released either through hormonal or electrical messages. It is located at the base of the brain and it is a hormone secreting gland compartmentalization into the anterior pituitary and posterior pituitary as is shown in figure (1-1) (Le et al., 2013)The anterior pituitary contains five major hormone secreting cell types. Corticotrophs produce adrenocorticotropic hormone (ACTH), gonadotrophs secrete follicle-stimulating hormone and luteinizing hormone, thyrotrophs secrete thyroid stimulating hormone, somatotrophs secrete growth hormone(GH) and lactotrophs secrete prolactin (PRL). (Dasen and Rosenfeld, 2001)differentiated five cell types that do not occur at the same time during development. Usually, the first differentiated cell is the corticotroph, followed by the differentiation of gonadotrophs, thyrotrophs, somatotrophs, and lactotrophs in that order (Gillam et al., 2006)

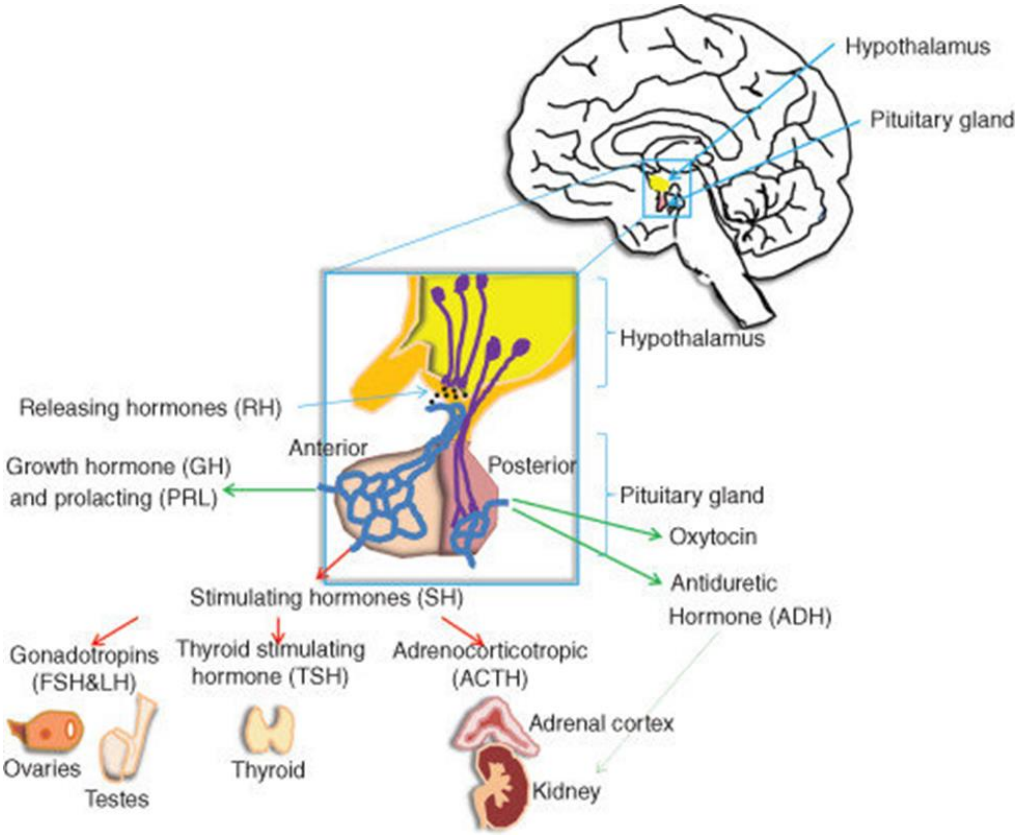


Figure (1-1):Endocrine system control(Kubba, 2016).

Although the anterior loop secretes at least eight hormones, only six have well established functions (Forsyth and Wallis, 2002).

- Growth hormone (GH):

It promotes growth in childhood. For adults, it helps to maintain healthy muscle and bone mass.

- Adernocorticotropic hormone (ACTH):

This hormone promotes the production of cortisol which helps to reduce stress, maintain healthy blood pressure and more sensation like hungry and thirsty.

- Thyroid stimulating hormone (TSH):

This hormone helps to regulate the body's thyroid, which is crucial in

maintaining a healthy metabolism T3, T4.

- •Luteinizing hormone (LH):

It is a glycoprotein hormone, and it is essential for reproduction females. Its function is to regulate estrogen, and at the time of menstruation, it initiates follicular growth, specifically affecting granulose cells (Olooto et al., 2012)But in men it regulates testosterone.

- Follicle stimulating hormone (FSH):

It is found in both men and women. It regulates the development, growth, pubertal maturation and reproductive processes of the human body.

In females, its function is to initiate follicular growth, stimulating the releasing of eggs, and in men it helps to ensure the normal functions of sperm production (Nore et al., 2013)

- Prolactin (PRL):

It is unique among the anterior pituitary hormones in which its major function is not to exert control over the secretion of a hormone by another endocrine gland. Its most important action is to stimulate the development of the mammary glands and milk production. It has direct effects upon the breasts (Kelley and Volpe, 2015)

The back part of the pituitary gland is called posterior pituitary. It produces the following two hormones:

- Oxytocin:

This hormone causes pregnant women to start having contractions at the

appropriate time. It also promotes milk flow.

- Antidiuretic hormone (ADH):

It is commonly referred to as vasopressin. This hormone helps to regulate water balance in the body. The hormones produced by the pituitary gland is shown in figure (1-2),(Kubba, 2016)

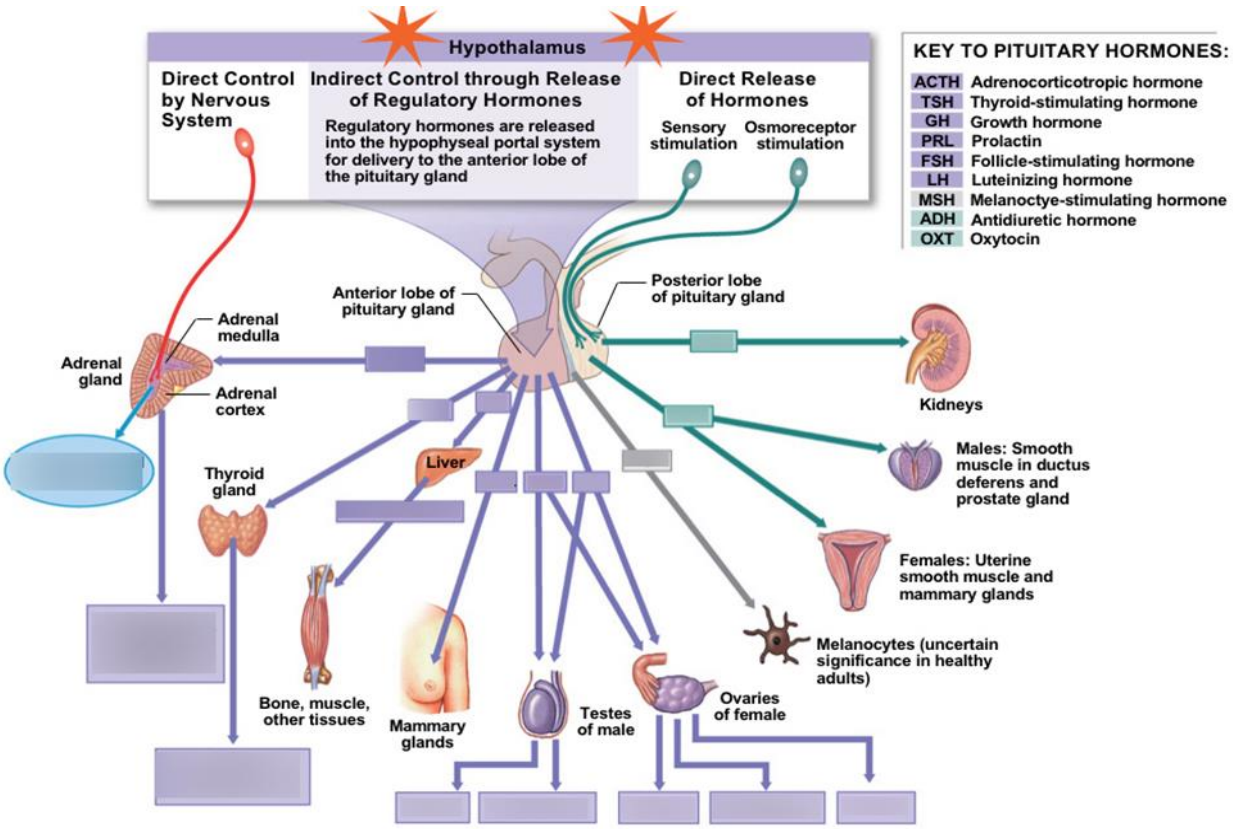


Figure (1-2): Hormones produced by the pituitary glands and their targets (Kubba, 2016).

1.2.3 Prolactin hormone.

1.2.3.1 Structure.

Prolactin hormone is a multifunctional hormone discovered by Stricker and Grueter as a pituitary factor that could induce milk secretion in rabbit mammary glands, and crop milk production in pigeons. The factor was purified and given the name prolactin shortly thereafter .The entire amino acid sequence, including a 28 residue single peptide, was discovered by a cook and his colleagues from the nucleotide sequence of human cDNA (Newey et al., 2013),Prolactin is a polypeptide hormone composed of 199 a.a (23KD), that is synthesized and secreted by specialized cells of the anterior pituitary gland (lactotroph) (Nitze et al., 2013),It circulates mainly in a monomeric form but variants of prolactin become of post translational modifications such as proteolytic cleavage, dimerazation, polymerization, phosphorylation and glycosylation (FUJIKAWA et al., 2000),The human prolactin circulates in blood in various sizes, monomeric PRL (little PRL 23KD), dimeric PRL (big PRL, 48-56 KD), and polymeric forms (big-bigPRL,100 KD). The monomeric form is the most bioactive PRL, and it has over 300 separate biological activates (Baban et al., 2008) ,In

general, these PRL variants have reduce the biological activity. Large molecular isoforms (>150 kD) are termed macroprolactin due to complexes of PRL and IgG (Davis, 2004) ,A variation in the levels of prolactin was noticed in mammals for instance during pregnancy. High circulating concentrations of estrogen and progesterone inhibit the action of prolactin on milk production. After delivery, reducing estrogen and progesterone production allows prolactin to induce lactation (Serri et al., 2003). The levels of prolactin after childbirth, fall as the internal stimulus for them are removed, but sucking by the baby on the nipple then promotes further prolactin release T h i s maintains the ability to lactate. The sucking activates mechano receptors in and around the nipple, then the signals are carried by nerve fibers through the spinal cord to the hypothalamus, where changes in the electrical activity of neurons that regulate the pituitary gland cause an increased prolactin secretion.The suckling stimulus also triggers the release of oxytocin from the posterior pituitary gland, which triggers milk let-down. Prolactin controls milk production (lactogenesis) but not the milk-ejection reflex; the rise in prolactin fills the breast with milk in preparation for the next feed (Nilsson and Hellberg, 2006).

1.2.3.2. Prolactin gene.

In mammals, the prolactin PRL gene family is a large family of paralogous genes encoding hormones and cytokines (Soares et al., 2007).The human prolactin gene is present as a single copy per haploid genome. It is located on chromosome 6 and divided into 5 exons and 4 introns (Sudmant et al., 2010)The molecular size of it is about 10.215 (kb) and the transcription is regulated by two independent promoter regions; the proximal 5000 bp region directs pituitary – specific expression , while more upstream (distal) promoter region is responsible for extrapituitary expression The human prolactin mRNA is 914 nucleotides long and contains a 618- nucleotide open reading frame translated prolactin prohormone of 227 amino acids. The 28 amino acid signal peptide is cleaved and the mature human prolactin is formed (199 amino acids)((Binart et al., 2010)

1.2.3.3. Prolactin receptor.

The actions of prolactin are initiated through an interaction with a specific cell surface high affinity prolactin receptor (PRLR)(Omelka et al., 2008) It is a member of the largest class-1-cytokine receptor super family(Li et al., 2006)Only a single isoform of the prolactin receptor has been identified in humans (Fayed, 2019)It is(Broutin et al., 2010) located on chromosome 5 and is approximately 180 kb in length and originally has 10 exons of which (3-10) coding exon(Sudmant

et al., 2010),(Taylor et al., 2007).The PRLR is composed of an extracellular ligand-binding domain which consists of 210 amino acid(Broutin et al., 2010). This receptor can be further divided into NH2-terminal D1, which has two pairs of disulfide bonds between cysteins (Cys12- Cys22 and Cys51-Cys62)and membrane proximal D2 domains which have conserved region "WS" motif(Trp-Ser-x-Trp-Ser). Both disulfide bond and "WS" motif are necessary for a proper folding and trafficking of the receptor. The second part consist of a transmembrane domain which is a 24 a.a hydrophobic domain and an intracellular domain which is essential for initiation of the signal transduction mechanisms associated with the prolactin receptor. The two intracellular conserved regions within the PRLR are termed Box1 which is a rich proline and it is necessary for the consensus folding of the molecule, and Box2 which is missing in a short isoform of the prolactin receptor (Newey et al., 2013, Nilsson and Hellberg, 2006).Receptors for prolactin are widely expressed in the mammary glands, ovaries pituitary glands, hearts, lung, thymus, spleen, liver, pancreas, kidney, adrenal glands, uterus, skeletal muscle and skin .This receptors has many actions in different tissues and this biological action happens because of the expression and regulation of different PRLR isoforms and the utilization of different signaling pathways (AbdElghani and Elmugadam, 2013)Like many other members of this family, the first step in receptor activation is generally believed to be a legend-induced dimerization whereby one molecule of PRL is bound to two molecules of receptor (Elkins et al., 2000). Recent reports suggest that PRLR pre-assembles at the plasma membrane in the absence of ligand (Elkins et al., 2000), suggesting that ligand-induced activation involves conformational changes in preformed PRLR dimers (Broutin et al., 2010). The genetic polymorphism in prolactin receptor genes can lead to a variation in plasma levels of encoded proteins (Dunning et al., 2004). 1.2.3.4. Regulation of prolactin hormone. (Mancini et al., 2008)A summary of the regulation of PRL secretion is presented in Figure (1-3)(Berinder, 2011).

1.2.3.4. Regulation of prolactin hormone.

Prolactin hormone synthesized and secreted from lactotroph cell, in the anterior pituitary, which compromises about 15-22% of functioning anterior pituitary cells (Morozova and Marra, 2008). The secretion of it is mainly under the tonic inhibition of hypothalamic dopamine(Dufour et al., 2023)Dopamine reaches the pituitary via the hypothalamic-pituitary portal system and inhibits PRL by binding to type 2 dopaminergic receptors on the lactotrophs leading to a rapid

suppression of PRL release from secretary vesicles, inhibition of PRL gene expression and lactotroph proliferation, PRL exerts a negative feedback on its own release by stimulating hypothalamic dopamine synthesis (Ben-Jonathan et al., 2002) Although the control of PRL secretion is mainly inhibitory, there are several known PRL-releasing factors, including thyrotropin releasing hormone (TRH), vasoactive intestinal polypeptide, oxytocin and endothelin. Also estrogens stimulate lactotroph cell proliferation as well as PRL secretion (Berinder, 2011). Moreover, estrogens activate secondary responses that may influence PRL gene transcription, i.e. inhibiting dopaminergic hypothalamic activity and upregulating TRH receptors. Furthermore, PRL secretion is increased by different forms of stressors. A summary of the regulation of PRL secretion is presented in Figure (1- 3),(Berinder, 2011).

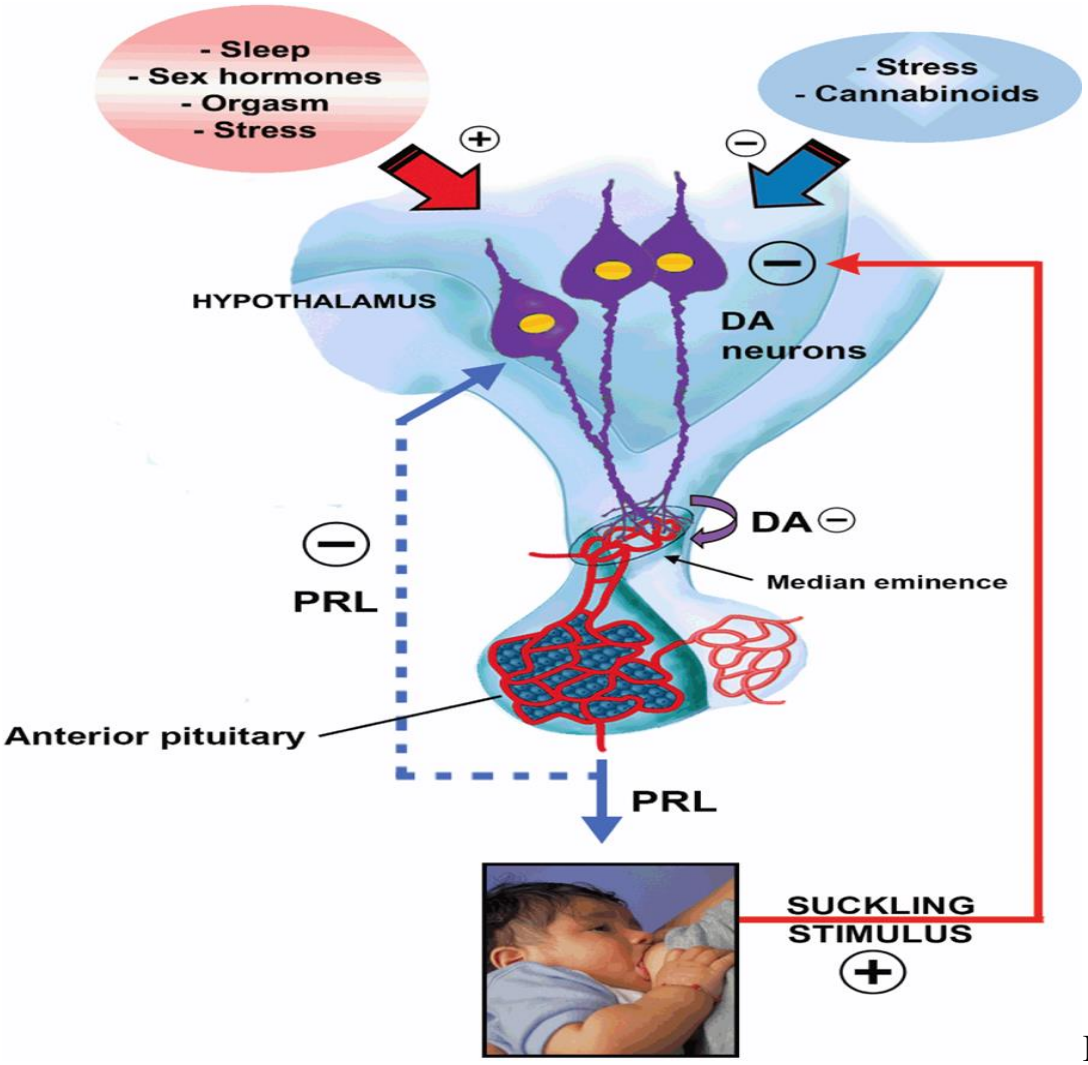
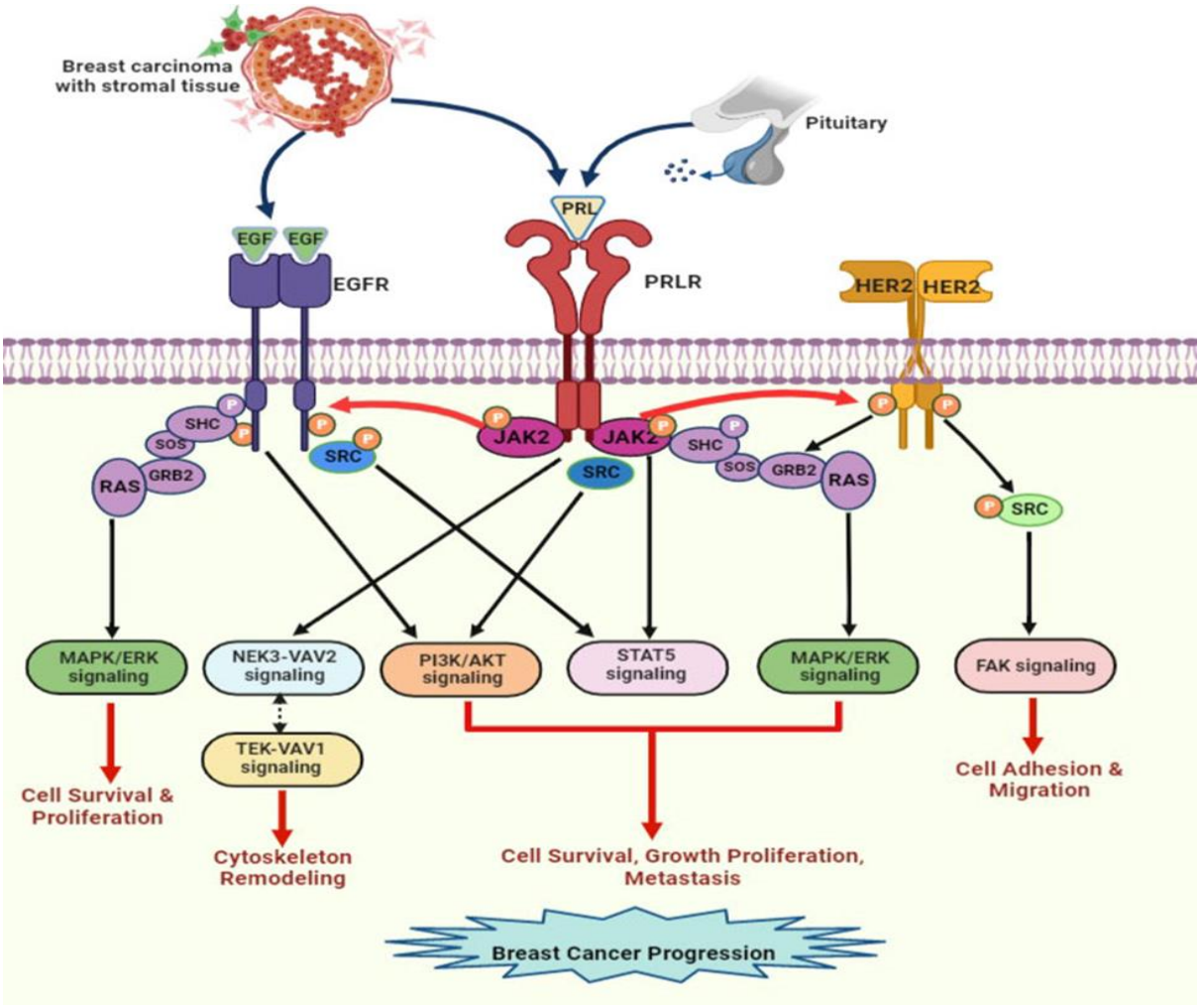


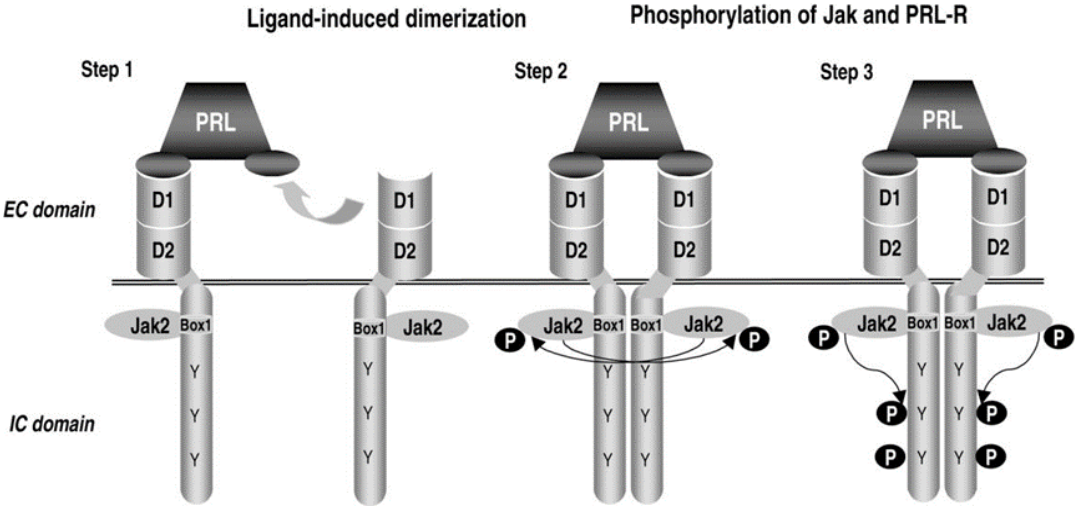
Figure (1-3): Regulation of prolactin secretion (Berinder, 2011)

1.2.4. Hormone signal transduction.

The function of PRL hormone in cells and tissues has been related to expression of PRLR on the cell surface and the utilization of signaling pathways. The PRLRs are non-kinase receptors whose activation of signaling pathways requires participation of receptor-associated kinases, such as Janus kinases or Src kinases (Ihle, 1994). The signal transduction of PRLR involves mainly JAK/Stat pathway as it is the most important signaling pathway used by cytokine receptors. The JAK2 activity, induced by PRLR dimerization, is necessary for PRL action (Olooto et al., 2012)

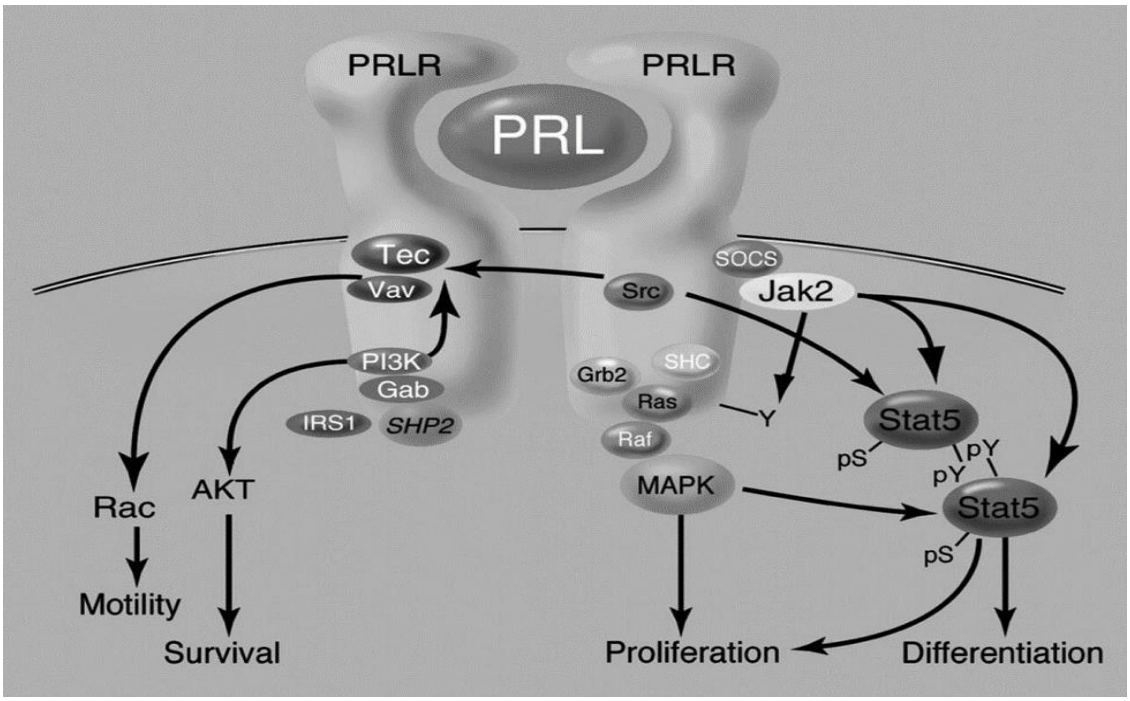
The PRL molecule contains two receptors-binding sites; PRL binding site 1 interacting first with one PRLR. This leads to a complex formation which is (PRL-PRLR), then PRL bind site 2 intracting with the second PRLR, resulting in a PRLR dimerazation and activation (Kubba, 2016), as shown in figure(1-4).





After a ligand stimulation of the receptor, JAK2 activation occurs within 1 min. The ligand-induced receptor dimerization will bring two receptors associated JAK molecules close together. This results in activation by transphorylation of JAK tyrosines. Activated JAK2 and phosphorylation of tyrosine residues are on PRLR. All PRLR isoforms can activate this JAK2 but

only tyrosine residues of the long and intermediate PRLR isoform are phosphorylated after JAK2 activation(Clevenger et al., 2003) The phosphorylation of tyrosine residues is important because it is considered as a potential binding site for transducers most of which contain Ser homology regions2 domains. This SCH2 domain is found in the signal transducer and activator of transcription (state) proteins as it contains DNA binding domain and a c-terminal trans activity domain. The state family consists of eight members. State 5 has two isoforms which are state 5a and state 5b, which are initially identified as PRL induced mammary gland transcription factors (Sudmant et al., 2010)The major difference between state5a and state 5b isoforms lies in their serine/threonine phosphorylation sites (Olooto et al., 2012)The SH domain of state interacts with the phosphorylated tyrosine residue of the activated long prolactin receptor isoform. This will make a complex PRLR/JAK2/STAT5, being stated with phosphorylate by the receptor associated with JAK kinase (Kubba, 2016).These phosphorylated states dissociate from the receptor and dimerize with the SH2 domain of another phosphorylated state molecule through their phosphorylation residues(Brooks, 2012),Finally, this dimer tranlocates to the nucleus and activates a stat DNA- binding motif in the promoter of a target gene (Omelka et al., 2008)The consensus DNA motif recognized by Stat1, Stat3, and Stat5 homo or heterodimers is termed GAS (gama_interferon activated sequence). GAS consists of a palindroic consensus sequence. Once bound, state engages several elements of the transcriptional machinery, stimulating gene expression(Fayed, 2019)



Apart from lactotrophs in the anterior pituitary, PRL is also produced by different organs and tissues. It is synthesized in many extra pituitary sites e.g., reproductive organs, immune cells and brain where it may function as an autocrine/ paracrine functions(Baban et al., 2008),(Ben-Jonathan et al., 2002)

1.2.5. Biological action of prolactin hormone.

More than 300 different biological functions have been attributed to prolactin (Baban et al., 2008). The actions of this anterior pituitary hormone in the body include immunoregulation and protection. This is because its widely recognized as an important physiological modulator of the immune response by acting in acytokine-like manner (Dorshkind and Horseman, 2000),(Clevenger et al., 2003). It stimulates T-cell proliferation (Clevenger et al., 2003) and supports interferon alfa production (Courtillot et al., 2010). Prolactin is synthesized and secreted by human peripheral blood mononuclear cells and it functions in an autocrine manner as a growth factor for lymphoproliferation (Kelley and Volpe, 2015).It is also involved in regulating monocyte/macrophage function in vitro (Aziz et al., 2008). Macrophage activation and superoxide anion production responsible for killing pathogenic organisms are effects mediated by the PRLR in inflammatory pathways(Omelka et al., 2008).In reproduction, the actions of PRL represent the largest group of different functions in which it exerts effects on the mammary gland development of females during pregnancy by stimulating the growth of it to allow the pregnant to prepare for breast feeding. This is one of the important things for the initiation and maintenance of lactation in the female (Banerjee et al., 2004, Blecher-Gonen et al., 2013). It also exerts effects on the targets important to the reproduction of the mammalian specie(Ben-Jonathan et al., 2002). It exerts effects on targets important to reproduction and many autocrine /paracrine functions (Fayed, 2019). PRL receptor is expressed in cells in testis prostate gland, seminal vesicles and ovary (Forsyth and Wallis, 2002) .The synthesis of milk (lactogenisis), stimulates the uptake of some amino acids, the synthesis of milk proteins casein and alfa lactoalbumin uptake of glucose and synthesis of milk sugar lactose also milk fat(Benker et al., 1990). Prolactin regulates a variety of brain functions including the suppression of adenocorticotrophin secretion during the stress response (Terasaki et al., 2010).One of the least understood actions of prolactin is the regulation of solute and water transport across mammalian cell membranes (Serri et al., 2003)Studies in this area were motivated by the finding in lower vertebrates that prolactin stimulates solute transport across cell membranes and thus could be an osmoregulatory hormone(Vyas, 2012) (Williams et al., 2003)summarizes the broad biological functions of PRL in five categories:

- water and electrolyte balance.
- growth and development.
- endocrinology and metabolism
- brain and behavior.
- reproduction.

1.2.6. Hyperprolactinemia.

A condition in which excess prolactin circulates in the blood stream of non lactating and non pregnant women and in males called "hyperprolactinemia" . Its probably one of the most common endocrine disorders related to pituitary function, and it is more commonly diagnosed in women than in men (Williams et al., 2003)

Normally prolactin is present in small amounts throughout the blood stream of non pregnant females and in males kept under control of another hormone called "prolactin inhibiting factor" (dopamine) (Fitzgerald and Dinan, 2008)in which the normal levels are typically 10-35 ng/ml in females and 5-10ng/ml in males, each 1 ng is equivalent to 21.2 mIU/ml(Clevenger et al., 2003). But there are numerous conditions that may cause elevated prolactin levels in females, such as secretion which may happen with physiological causes, such as increases mildly with sleep, stress, exercise, nipple stimulation, lactation and pregnancy(Jacobson et al., 2011). Besides, there are a pathological reasons that cause prolactin secretion increase the hypothalamic disorders such as tumors, or infiltrative disease like tuberculosis. It may also relate to pituitary disorders such as prolactinoma, macroadenoma, or may be of other reasons such as polycystic ovarian disease, primary hypothyroidism, chronic renal failure, liver cirrhosis or some medication(Mancini et al., 2008). Hyperprolactinemia may result in hypogonadism, infertility, and galactorrhea, or it may remain a symptomatic (Gillam et al., 2006), (Kester et al., 2004).

The signs of hyperprolactinemia are represented by irregular menstrual, milky discharge from the breast (amenorrhea), headache, sometimes change in vision also estrogen level can be decreased to a point where the loss of bone calcium can occur (Morozova and Marra, 2008).

CHAPTRE TWO

2.1. Biochemistry and physiology

Prolactin is a protein consisting of 197–199 amino acids, and is structurally similar to growth hormone and human placental lactogen. The three hormones are believed to originate from a common ancestral protein created 60 million years ago. Prolactin has various functions in different species, but there are many homologous coding sequences in the prolactin gene that have been conserved throughout evolution (Glass and Kase, 1991). Prolactin is coded by one single gene, but the molecule is still heterogeneous because of posttranscriptional and posttransla- tional modifications including polymerization, gly- cosylation, and phosphorylation. The main part of PRL is nonglycosylated (75%). This proportion in- creases even more during pregnancy as well as in the case of prolactinomas. The reason for this is unknown (Brue et al., 1992). There are at least five isoforms of PRL. This has complicated earlier radioimmuno- assays where polyclonal antibodies were used. To- day more specific tests with monoclonal antibodies are used. The major circulating form of PRL is a polypeptide of 23 KDa. There is also an immuno- reactive 'big' prolactin (50 kDa), which is a dimer, and a macroprolactin, 'Big, Big' prolactin (160 kDa). Previously, it was believed that these large forms were oligomers of PRL. Recent studies have shown that they are antigen-antibody complexes of PRL and immunoglobulin G. In macroprolactin- emia, a new syndrome, one can find patients with elevated PRL levels consisting mainly of this mac- roprolactin, which has low bioactivity. These pa- tients usually lack clinical symptoms. These anti- gen-antibody complexes, however, can be unstable and dissociate, giving rise to symptomatic hyperprolactinemia. The same mechanism can be seen during periods with low insulin levels, when complexes of insulin and insulin antibodies can dissociate and cause hypoglycemia. There are sev- eral known cases with markedly elevated PRL levels, yet without any clinical symptoms and with no evidence of a pituitary adenoma. They are in- sensitive to dopamine agonist treatment, and ex- tended investigation shows that their prolactin iso- form is mainly macroprolactin. This should be considered in cases where laboratory results do not match clinical findings. The cause of this antibody formation is unknown. It can be seen in cases of lymphocytic hypophysitis but is otherwise not as- sociated with prolactinomas, other CNS tumors, or pregnancy (Brue et al., 1992, Lindstedt, 1994, Hattori et al., 1992). Prolactin is mainly secreted

by the lactotroph cells in the anterior pituitary gland. There is also production in decidualized endometrium and in the myometrium. During pregnancy the concentration of PRL rises in the amniotic fluid, therefore this decidual prolactin may be important for fluid and electrolyte regulation. PRL reduces the permeability of the fetal membranes in the fetal to maternal direction. Levels of amniotic PRL are lower in pathologic pregnancies such as preeclampsia and polyhydramniosis, but this seems to have no clinical signifi- cance. Prolactin produced by the fetal pituitary acts as an antidiuretic hormone and to some extent regulates fetal water and electrolyte balance. Bro- mocriptine treatment during pregnancy suppresses maternal and fetal prolactin production but does not affect amniotic prolactin or fetal growth an

2.2. Pathologic hyperprolactinemia

Serum prolactin should routinely be assayed in pa- tients being treated for infertility, menstrual dis- orders, galactorrhea, and pituitary adenomas ((Sarapura and Schlaff, 1993), (Batrinos et al., 1994). The test is done in the morning or afternoon at least one hour after a meal. Considering the pul- satile secretion, a slightly elevated level should be confirmed with repeated samples (AbdElghani and Elmugadam, 2013). The upper limit of the normal value for serum prolactin varies among different authors. However, if the upper limit is set at 20 ng/mLΩ470 mU/L (PRL ng/mL¿23,3ΩmU/L), one will have a bell-shaped dis- tribution of values including 97% of women with and without menstrual disorders within this range (Broutin et al., 2010). Values between 16 and 20 ng/mL represent the upper extreme encountered by only a small percentage of these women. Prolactin values from 21 to 30 ng/mL should be confirmed in a repeated sample but otherwise warrant the usual search for a prolactinoma even if this is extremely rare in this range. With moderate hyperprolactinemia (31–49 ng/mL), the incidence of microadenomas is higher, especially when associated with galactorrhea or menstrual irregularities (60%). The incidence of hyperprolactinemia in women with secondary amenorrhea is 10%. The majority of these women have values above 30 ng/mL, suggestive of a pro- lactinoma. In this study of 4199 women, the preva- lence of prolactinomas was 3%, which is in accord- ance with earlier population studies (Broutin et al., 2010), (Stewart et al., 1993).

2.3. Evaluation of hyperprolactinemia

With a careful history, physical examination, thy- roid function tests, and a pregnancy test, one may exclude virtually all other causes except for hypothalamic-pituitary disease. A full endocrine assessment is made initially. Measurements of TSH, growth hormone, ACTH, and urinary free cortisol will reveal a TSH-secreting tumor, acro- megaly, or Cushing's disease. To evaluate the presence of hypothalamic-pituitary disease com- puted tomography (CT scan) or magnetic reson- ance imaging (MRI) and visual field examination should be performed. Evaluation with pituitary imaging is essential even in women with only mild PRL elevation (20–30 ng/mL), in order not to overlook a pseudoprolactinoma or nonsecret- ing tumor. These only rarely respond to bromoc- riptine (10–50%), and left untreated there is risk for continued growth and compression of the op- tic chiasm (Kubba, 2016), (Stewart et al., 1993). Visual field examinations by Goldmann perimetry are done on patients with suprasellar extension or tumor growth less than 2 mm from the optic chiasm (Kubba, 2016), (Blackwell, 1992). Most 'nonse- creting adenomas' are so-called gonadotropinom- as but still secrete FSH, a-subunit, or LH. A-sub- unit can be used as a tumor marker except in postmenopausal women because of the increased secretion that accompanies the secretion of gonadotropins (Berinder, 2011). Dynamic stimulation and sup- pression tests with TRH, chlorpromazine, and L- dopa have been abandoned because of inconclus- ive results (Blackwell, 1992).

2.4. Symptoms

The most common symptoms in women are sec- ondary amenorrhea, decreased libido, menstrual disorders, and galactorrhea. Galactorrhea is defined as a nonphysiologic sec- retion from the breast that is not related to preg- nancy or lactation. Other breast diseases often give discoloured secretion from a single duct. Galac- torrhea can be seen with all the different con- ditions that give rise to hyperprolactinemia, but it also occurs in women with normal PRL levels. In these women, it is believed to be caused by an in- creased sensitivity to PRL in the breast or, alterna- tively, by a transient hyperprolactinemia (see be- low). Fortunately, long-standing galactorrhea on the basis of chronically elevated PRL concen- trations does not seem to increase the risk of breast cancer. On account of low estrogen levels, less than half of the women with hyperprolactinemia also have galactorrhea. Treatment can be given with dopamine agonists and is effective even with nor- mal PRL levels ((Blackwell, 1992), (Binart et al., 2010), (Blecher-Gonen et al., 2013). Hypogonadism

caused by hyperprolactinemia can lead to infertility but also osteoporosis and possibly cardiovascular disease on account of low estrogen levels. Most commonly a disease of adult life, hyperprolactinemia can be seen in preado- lescent children and give rise to primary amenor- rhea. Macroadenomas can cause headaches and visual field impairment, as well as hypopituitarism secondary to tumor pressure on surrounding struc- tures , (Blackwell, 1992, Banerjee et al., 2004),(AbdElghani and Elmugadam, 2013).

2.5. Causes of hyperprolactinemia

Pathologic hyperprolactinemia can have many dif- ferent causes. The most common are prolactinom- as, hypothyroidism, and treatment with psycho- tropic drugs.

2.5.1. Antipsychotic

Antipsychotic drugs can block dopamine receptors on the pituitary lactotrophs and prevent the normal dopamine-mediated inhibition of PRL release. There is a dose-response relationship between the amount of antipsychotic drug given and the level of increase in PRL. This increase usually levels out after the first week of treatment. During chronic treatment, PRL levels usually remain elevated but to a lesser degree. Normalization occurs in about 30% of treated women. After discontinuation of therapy with antipsychotics, PRL levels usually return to normal within a couple of days. Galactorrhea is the most troublesome symptom that can affect more than half of the patients treated, usually premeno- pausal women on account of higher estrogen levels. Reduction of the antipsychotic dosage or, if poss- ible, discontinuation of therapy is the treatment of choice if symptoms occur. Changing to another drug is not likely to succeed because of similar ad- verse effects. Clozapine seems to affect PRL con- centrations the least. Bromocriptine has been tried in symptomatic patients who require continued treatment with antipsychotics. It improves galac- torrhea, even if it does not eliminate it completely. As a result of low doses, adverse psychiatric effects of bromocriptine seldom occur. Treatment with antidepressants, benzodiazepines, and lithium can cause modest hyperprolactinemia but seldom any clinical symptoms. Other possible causes must be investigated (Marken et al., 1992).

2.5.2 Hypothyroidism

Primary hypothyroidism can cause a moderate hyperprolactinemia through increased TRH levels. Patients with long-standing hypothyroidism and an enlarged pituitary as well as symptomatic hyperpro- lactinemia, may be misdiagnosed as having a pro- lactinoma. Substitution with thyroxine will normal- ize PRL levels and the pituitary size.

2.5.3 Additional causes

There is also a relatively high coincidence of hyperprolactinemia and polycystic ovary disease (30%). Chronically increased estrogen levels are as- sumed to be responsible for this. Hyperprolac- tinemia in these patients is usually moderate and esponds to bromocriptine (Kubba, 2016). Cirrhosis and re- nal failure are also associated with hyperprolac- tinemia caused by altered hypothalamic regulation of PRL. Ectopic production from a carcinoma of the lungs or kidneys, ovarian teratoma, or myoma occurs sporadically (Rui, 2000), (Vyas, 2012). Hypothalamic tumors (craniopharyngiomas) and other diseases such as sarcoidosis and eosinophilic granuloma, which dis- tort and compress the pituitary stalk, are believed to cause hyperprolactinemia through decreased PIF (dopamine) transmission but with continued PRF activity. This is supported by the observation that patients with complete stalk interruption are more inclined to have lower PRL levels than pa- tients with only stalk compression (Williams et al., 2003).

2.5.4. Transient hyperprolactinemia

Transient hyperprolactinemia has been described as the mildest form of disease that causes infertility through luteal insufficiency. A subgroup of infer- tile patients with an exaggerated response of PRL to TRH have been shown to have galactorrhea, lu- teal insufficiency, and anovulatory cycles. These patients have high basal levels of PRL as well as nocturnal and transient hyperprolactinemia dur- ing the menstrual cycle (Fig. 3). A possible mech- anism could be an increased receptor sensitivity to PRL centrally in the hypothalamus-pituitary or peripherally in the breast and ovary (Sudmant et al., 2010), (Terasaki et al., 2010), (Kester et al., 2004). Previously, euprolactinemic anovulatory women were given bromocriptine more or less empirically which supposedly improved pregnancy rates. Re- cent controlled studies have demonstrated that nothing is gained by giving bromocriptine to ovu- latory women with unexplained infertility. In ro- dents,

PRL inhibits the conversion of androgens to estrogens by blocking the aromatase enzyme on the surface of granulosa cells, which causes the hy- poestrogenism that is seen with hyperprolac- tinemia (Hattori et al., 1992). In humans, the situation is more com- plex. Physiologic levels of PRL seem to be neces- sary for normal luteinization (see above) (Gillam et al., 2006), whereas high levels inhibit both progesterone and estradiol synthesis. There is a four to sixfold higher concentration of PRL in follicles compared to serum. If serum levels exceed 100 ng/mL, all fol- licles become atretic. Previous studies have shown that follicles are most sensitive to excess PRL dur- ing the recruitment period. Iatrogenically induced hyperprolactinemia using metoclopramide, a dop- amine receptor antagonist given during the luteal phase, does not seem to affect the luteal phase or progesterone levels. The main reason why hyperprolactinemia causes anovulation and low es- trogen levels is hypothalamic, since hyperprolac- tinemia induces increased levels of dopamine that suppress the pulsatile secretion of GnRH (Glass and Kase, 1991). In conclusion, it is difficult to determine whether or not there is a condition of transient hyperprolac- tinemia that can cause infertility. Occasionally ele- vated PRL levels should be interpreted cautiously. Euprolactinemic women with galactorrhea require extended evaluation and treatment.

2.5.5.Idiopathic hyperprolactinemia

When no specific cause is found, the hyperprolactinemia is called idiopathic. Many cases are caused by a microadenoma that is not visible with current imaging techniques. A CT scan cannot distinguish small microadenomas (∞2 mm) from normal pitu- itary tissue. With MRI the specificity is very high (90–100% true-positive confirmed by later surgery). However, the sensitivity of the MRI is not yet defined. Idiopathic hyperprolactinemia can constitute more than a third of the total number of patients with hyperprolactinemia. Many of these patients exhibit symptoms (Hattori et al., 1992). Long-term follow- up shows that one third recover spontaneously and about 10–15% progress to a microadenoma within 2–6 years (FUJIKAWA et al., 2000). In conclusion, the condition is self- limiting and probably has a pathophysiology dif- ferent from the pathophysiology of adenomas. Resolution is not affected by either treatment with bromocriptine or pregnancy. CT should be done in the initial evaluation or before a planned preg- nancy. Annual measurements of PRL levels are otherwise sufficient. Treatment is given to patients with troublesome galactorrhea or anovulatory in- fertility and also to prevent osteoporosis in con-

nection with hypogonadism (Glass and Kase, 1991), (Fitzgerald and Dinan, 2008). Another cause of unexplained hyperprolactinemia is macroprol- actinemia, i.e., the presence of PRL antibodies that form a macroprolactin with low bioactivity and only slight symptoms (see above) (Morozova and Marra, 2008), (Morozova and Marra, 2008), (Le et al., 2013).

2.6.Prolactin adenomas

Prolactinomas are the most common endogenous cause of hyperprolactinemia. The disease can, however, be caused by any central nervous system tumor that interferes with the tonic inhibitory con- trol of dopamine from the hypothalamus. The ex- act histopathological diagnosis can only be ob- tained through surgery. As long as the adenoma responds to dopamine agonist therapy, there is no need to secure a diagnosis. Prolactinomas are di- vided into microadenomas (∞10 mm) and mac- roadenomas. In general, there is a correlation be- tween adenoma size and serum prolactin levels. This relationship, however, is not absolute. Large tumors with rather low prolactin levels are often non-prolactin secreting adenomas causing pitu- itary stalk compression. The true prevalence of prolactinomas is still debated. In earlier studies based on autopsies, microadenomas have been found in 27% of women, of which 40% have been prolactinomas based on immunocytochemistry. Most of these have been of microscopic size, not de- tectable with current imaging techniques and re- garded as accidental findings. The prevalence of de- tectable (±3 mm) adenomas is stated to be 3% in more recent population studies (Davis, 2004), (Dufour et al., 2023). Prolactin- omas, regardless of size, have a greater prevalence in younger age groups. More women than men are affected, possibly through the stimulatory effect of estrogen. Macroadenomas are less common but often larger in men, which is probably due to their longer duration before symptoms appear. The etiol- ogy of prolactinomas is not fully known. One theory suggests that it is caused by a mutation of the D2 re- ceptor coding genes on chromosome 11 (Fang et al., 2010). This would then lead to a change in structure or function of the dopamine receptor and lead to a deficient or absent inhibitory effect of DA on PRL secretion. Prolactinomas are seldom malignant but still show signs of neoplasia. Cell culture studies of adenoma cells have shown that these cells often are mono- clonal. This would support the etiologic theory of a somatic mutation **Pseudoprolactinoma** : This is a pituitary tumor that does not secrete PRL but still causes hyperprolac- tinemia through stalk compression. The wide- spread use of CT scans has caused a

number of ‘Incidentalomas’, i.e., clinically and biochemically inactive, non-progressive pituitary adenomas that are discovered accidentally. Previously, these pa- tients were often subject to surgery, but more re- cent studies have shown that the vast majority of both micro- and macro-incidentalomas do not pro- gress, at least not without symptoms (Jacobson et al., 2011). The course resembles that of a microadenoma. Alter- native diagnoses are pituitary adenoma, meta- stasis, glioma, craniopharyngeoma, infarct, men- ingioma, sarcoidosis, aneurysm, and hypophysitis.

2.7.Functional hyperprolactinemia:

Pituitary enlarge- ment caused by hyperplasia without an adenoma is uncommon but can be seen in untreated primary hypothyroidism, with GH secreting tumors, during pregnancy, and in functional hyperprolactinemia. This is a condition with widely fluctuating PRL levels from day to day and a marked increase of PRL in response to TRH. Should the patient be operated on suspicion of an existing adenoma, only lactotroph hyperplasia at the histopatholog- ical level will be found. It is important to distin- guish this condition from a prolactinoma. With PRL secreting adenomas, the levels of PRL are constantly elevated, the response to TRH is usually blunted, the pituitary stalk is often asym- metrically dislocated from the midline, and there is an inhomogeneous signal intensity on CT. The etiology is unknown, but an imbalance of estradi- ol-progesterone has been implicated. Further pro- gression to adenoma has been seen in rodents but not in humans (Soares et al., 2007).

Conclusions

Hyperprolactinemia is a common disorder with a variety of causes that can occur in all age groups. The condition can cause infertility but also many other symptoms and requires evaluation and treat- ment throughout a lifetime. During recent years, an increased understanding about the pathophys- iology has been achieved, but the etiology of pro- lactinomas remains unclear. The development of new, more potent dopamine agonists has made it possible to control symptoms and to correct hypo- gonadism and infertility in almost all patients. There are several new trends concerning future treatment of hyperprolactinemia. The first is a more conservative attitude towards treatment of moderate hyperprolactinemia with regard to the high degree of spontaneous resolution and the minimal risk of tumor progression. Further, an in- creased use of estrogen replacement therapy for hypogonadal patients who do not desire a preg- nancy. Finally,

the use of surgery as a complement to medical treatment for slow responders and pa- tients who wish to avoid lifelong treatme

CHAPTER FOUR

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