Unit 14

HORMONE ASSAYS

Structure

14.1 INTRODUCTION

Hormone play important roles throughout our life by controlling almost every process in our body. Changes in their levels therefore affect the processes in which they participate causing development of diseases that manifest into number of symptoms. Hormone assays are vital for the analysis of metabolic processes and various clinical conditions arising due to hormonal imbalances as well as for monitoring the efficacy of treatment. In this unit, we shall briefly summarize the clinical conditions associated with different hormones. We shall also describe the clinical relevance of thyroid stimulating hormone and human chorionic gonadotropin hormone. Principle of the assay used for determination of their levels will also be discussed.

Expected Learning Outcomes

After studying this unit, you should be able to:

- Enlist the importance of hormones imbalance as marker of certain diseases;
- explain the clinical conditions associated with imbalance of thyroid hormones;
- name the thyroid function tests and their interpretation; and
- explain the clinical significance of hCG hormone.

14.2 HORMONES ASSOCIATED TO CLINICAL CONDITIONS

Hormones are the chemical messengers that participate in wide range of activities ranging from birth, growth and development, metabolism, reproduction, mental health to aging. Imbalances in hormone levels can, therefore, lead to a range of health issues, making the study of hormones crucial for diagnosis and treatment of various conditions. Hormonal imbalance disorders occur when there's either too much or too little of a specific hormone in the body, disrupting the body's normal functions. Common examples include diabetes, thyroid disorders (hyperthyroidism and hypothyroidism), and conditions like PCOS (Polycystic Ovary Syndrome). Table 14.1 lists some of the disorders due to hormonal imbalance.

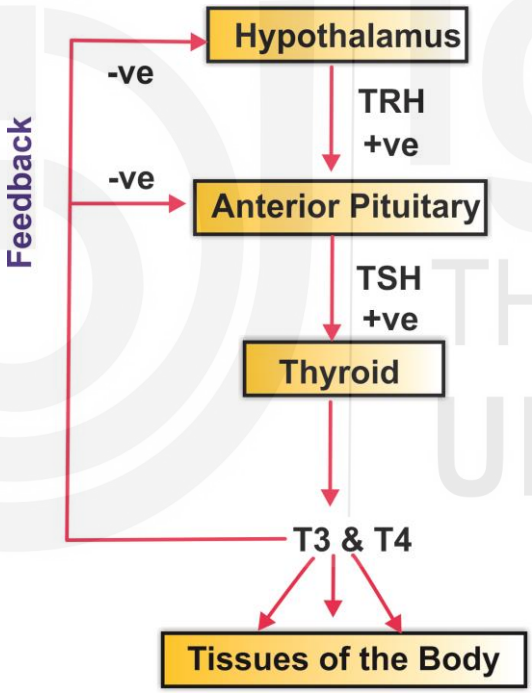
Table 14.1: Commonly known disorders due to hormonal imbalances

Disorder	Hormone	Clinical implications
Diabetes	Low levels of insulin	Increase blood glucose levels
Hyperthyroidism	Excessive production of thyroid hormones	weight loss, rapid heartbeat, and anxiety
Hypothyroidism	Underproduction of thyroid hormones	fatigue, weight gain, and depression
Addison's Disease	Insufficient production of cortisol and aldosterone by the adrenal glands	fatigue, weight loss, and low blood pressure
Cushing's Syndrome	Excessive secretion of cortisol	weight gain, muscle weakness, and thinning skin
Diabetes insipidus	Decreased Antidiuretic Hormone	inability of the kidneys to concentrate urine
Gigantism	Excess GH in childhood before the growth plates in bones have fused	Abnormally tall stature, enlarged hands and feet, thickened facial features (like a prominent forehead and jaw), and delayed puberty
Acromegaly	Hypersecretion of growth hormone in adults after bones have fused	enlarged bones in the hands, feet, and face, and other soft tissue changes, but not increased height.
Polycystic Ovary Syndrome (PCOS)	Imbalance of reproductive hormones	irregular or absent menstrual periods, cysts in ovaries of some women

All hormones play role in maintaining the health and wellbeing of an individual, thyroid hormone affects virtually every organ system in the body, including the heart, CNS, autonomic nervous system, bone, GI, and metabolism. Therefore, in this unit, we shall discuss about their clinical significance in detail.

14.3 THYROID HORMONES

The thyroid gland constitutes part of hypothalamic-pituitary-thyroid axis referred to as a self-regulatory circuit. Follicular cells of thyroid gland secrete two hormones; thyroxine or tetraiodothyronine (T4) and triiodothyronine (T3). Since both these thyroid hormones are lipophilic, these are transported in blood bound to various transporters. These include thyroxine-binding globulin (TBG), transthyretin, and albumin. Only a fraction (approximately 0.2%) of the thyroid hormone is free and active (free T4).When transporter bound hormone reaches its target site, T3 and T4 dissociate from their transporter protein to enter cells either by diffusion or carrier-mediated transport. T3 or T4 then bind to nuclear alpha or beta receptors in the respective tissue and cause activation of transcription factors leading to the activation of certain genes and cell-specific responses. Thyroid hormones are degraded in the liver via sulfation and glucuronidation and excreted in the bile.



C- cells (also known as parafollicular cells) of thyroid glands secrete another hormone; calcitonin that influences

Fig 14.1: Hypothalamic-pituitary-thyroid axis and secretion and regulation of thyroid hormones by feedback mechanism.

Thyroid hormones are secreted when stimulated by TSH by secreted by the anterior pituitary gland that in turn is stimulated by the thyrotropin releasing hormone (TRH) of hypothalamus (Fig .14.1). Thyrotropin-releasing hormone (TRH) from the hypothalamus, thyroid-stimulating hormone (TSH) from the anterior pituitary gland, and T4 from thyroid gland work in synchronous harmony to maintain the proper feedback mechanisms and homeostasis.In addition to TRH and TSH, availability of iodine also affects T3 and T4 synthesis.

Increased free T4 and T3 inhibit the release of TRH and TSH through a negative feedback loop. As a result, T3 and T4 secretion and iodine uptake

are reduced. Other hormones, such as somatostatin, glucocorticoids, and dopamine, also inhibit TSH production. Cold, stress, and exercise increase TRH release.

Let us have a look at the physiological functions of thyroid hormones:

- Increases the basal metabolic rate
- Depending on the metabolic status, it can induce lipolysis or lipid synthesis.
- Stimulate the metabolism of carbohydrates
- Activate anabolism of proteins. Thyroid hormones can also induce catabolism of proteins in high doses.
- Permissive effect on catecholamines resulting inincreased expression of beta-receptors to increase heart rate, stroke volume, cardiac output, and contractility.
- In children, thyroid hormones act synergistically with growth hormone to stimulate bone growth.
- During the prenatal period, thyroid hormone is needed for the maturation of the brain. In adults, it can affect mood. Hyperthyroidism can lead to hyperexcitability and irritability. Hypothyroidism can cause impaired memory, slowed speech, and sleepiness.
- Thyroid hormone affects fertility, ovulation, and menstruation by permissive effect on the action of reproductive hormones.

Thyroid gland malfunction may manifest as hypothyroidism or hyperthyroidism, which may be of primary (due to abnormality in thyroid gland) or secondary (due to abnormality pituitary) or tertiary (due to hypothalamic abnormality) origin.

Let us understand the pathophysiology of theroid hormones.

14.3.1 Clinical Significance

Hyperthyroidism: In primary hyperthyroidism, hyperactivity of the thyroid gland can result in excess T3 and T4 production along with the compensatory decrease of TSH. Secondary cause of T3 and T4 production is thyrotroph adenoma.It a rare type of pituitary tumor that secretes excess thyroid-stimulating hormone (TSH), leading to hyperthyroidism. Sometimes ectopic location of thyroid glands may lead to its overactivity.

Generalized hypermetabolism from hyperthyroidism causes increased Na+/K+-ATPase to promote thermogenesis. There is increased catecholamine secretion, and beta-adrenergic receptors are also upregulated in various tissues. As a result of the hyperadrenergic state, peripheral vascular resistance is decreased. In the heart, hyperthyroidism causes a decreased amount of phospholamban, a protein that normally decreases the affinity of

calcium-ATPase for calcium in the sarcoplasmic reticulum. As a result of decreased phospholamban, there is increased Ca+ movement between the sarcoplasmic reticulum and cytosol, leading to increased contractility. Increased beta receptors in the heart also lead to increased cardiac output.

Hypothyroidism: In primary hypothyroidism, decreased production of thyroid hormones by the thyroid gland causes a compensatory increase of TSH. Secondary hypothyroidism is caused by pituitary disorders causing decreased TSH release and decreased T3/T4 levels. Tertiary hypothyroidism is caused by hypothalamic disorders, resulting in decreased TRH levels, decreased TSH, and T3/T4 levels.

Ectopic thyroid refers to thyroid tissue located outside of its normal anatomical position in the neck. It arises from a developmental abnormality where the thyroid gland fails to migrate from its origin at the base of the tongue to its usual location in front of the trachea. This tissue can be found anywhere along the path of the thyroglossal duct, from the base of the tongue to the mediastinum.

Generalized symptoms associated with decreased activity of thyroid glands include slow basal metabolic rate that can present as apathy, slowed cognition, skin dryness, alopecia, increased low-density lipoproteins, and increased triglycerides. Therefore, in psychiatric patients presenting apathy and slowed cognition, hypothyroidism must be ruled out. Hypothyroidism can decrease sympathetic activity leading to decreased sweating, bradycardia, and constipation. Myopathy (muscle weakness) and decreased cardiac output because of decreased transcription of sarcolemma genes are also reported in some patients.

Thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates prolactin and TSH release. Thus, hypothyroidism may lead to hyperprolactinemia.More prolactin release can suppress testosterone, LH, FSH, and GnRH release and promote breast tissue growth.

Myopathy is a general term for diseases that affect the muscles that control voluntary movement, leading to muscle weakness.

Patients with hypothyroidism may present with myxedema in which there is swelling of the skin and underlying tissues giving a waxy consistency. It is caused by the decreased clearance of complex glycosaminoglycans and hyaluronic acids from the reticular layer of the dermis. Initially, thepretibial nonpittingedema is seen (Fig. 14.2), however, with untreated condition, it may develop into generalized edema.

Fig. 14.2: Pretibial edema in hypothyroidism.

Graves disease and Hashimoto disease are the most common causes of hyperthyroidism and hyperthyroidism, respectively.

Graves Disease

Fig 14.3: Red bulging eye in Graves disease.

Graves disease is the most common cause of hyperthyroidism. It is an autoimmune disease characterized by the production of TSH receptor antibodies, increasing the TSH levels.Due to increased TSH, these patients have abnormally high T4 and T3 levels.The disease can be diagnosed by a positive TSH-receptor IgG immunoglobulin test. Immunoglobulin G (IgG) against TSH-receptor leads to increased thyroid function and growth. Symptoms of hyperthyroidism and diffuse goiter are present. TSH-receptor antibodies can also activate orbital fibroblasts leading to fibroblast proliferation and differentiation to adipocytes. As a result, there is increased production of hyaluronic acid and glycosaminoglycan (GAG), leading to an increased volume of intraorbital fat and muscle tissue. It causes exophthalmos, lid retraction, and diplopia due to ocular motility problems. Eyes are red and bulging (Fig 14.3). Pretibial myxedema is characteristic finding in Graves' disease. It is due to the stimulation of dermal fibroblasts that leads to depositions of GAGs in the connective tissue. 70% of patients with Graves disease have elevated anti-TPO antibodies.

Hashimoto Thyroiditis

It is the most common cause of hypothyroidism in iodine-sufficient areas and is caused by autoimmune-mediated destruction of the thyroid gland. CD8+ T-cells cause thyroid follicular cell death. The release of IFN-gamma by TH1 cells causes the recruitment and activation of macrophages. During the early stage of the disease, the patient may develop a non-tender, symmetrical, and painless goiter. As inflammation continues, thyroid follicles are damaged and can rupture. When thyroid follicles rupture, the patient may be asymptomatic or can experience Hashitoxicosis (thyroid hormone from ruptured follicles, causing symptoms of hyperthyroidism). As the disease progresses, the thyroid gland may become normal-size or small, depending on the extent of fibrosis. As a result, the patient can develop the symptoms of hypothyroidism. In addition to cell-mediated destruction, anti-thyroid autoantibodies (anti-thyroglobulin and anti-TPO) are also produced, leading to antibody-dependent cell-mediated cytotoxicity. Hashimoto thyroiditis is diagnosed via ultrasound, antibody detection, and thyroid function testing. Radioactive iodine uptake test and fine-needle aspiration can be performed to exclude malignancy.

14.3.2 Diagnosis of Thyroid Function

Two types of thyroid tests are advised: blood and imaging tests. Blood tests generally include testing for TSH, T3 and T4, and thyroid antibodies.The initial tests of choice to screen for any thyroid abnormality are a TSH and a free thyroxine (free T4) test. These determine whether the abnormality arises centrally from the thyroid gland (primary), peripherally from the pituitary (secondary), or hypothalamus (tertiary). In primary hypothyroidism is suspected, the thyroid gland is not releasing enough thyroid hormones. Therefore, TSH levels will be appropriately elevated, while free T4 levels will be lower. In primary hyperthyroidism, free T4 levels are abnormally increased, and TSH levels will be appropriately decreased. Other lab tests such as TSH receptor antibodies or antibodies to thyroid peroxidase can help aid in

diagnosing Graves disease or Hashimoto thyroiditis, respectively.Normal thyroglobulin values indicate intact thyroid; its low or undetectable values are used as an indicator of successful thyroidectomy. Their high levels are present in case of iodine deficiency while rising levels indicate cancer recurrence or spread in patients with history of thyroid cancer. Table 14.2 provides an overview of thyroid function tests done in a biochemistry lab and normal range values of each parameter.

Table 14.2: Parameters determined for thyroid function

Parameter	Normal range
TSH (thyroid-stimulating hormone).	0.4 and 4.0 mIU/L
T3 or free T3 (tri-iodothyronine).	80 and 200 ng/dL
T4 or free T4 (thyroxine).	4.5 and 12.0 mcg/dL
TPO (thyroid peroxidase antibodies), also known as microsomal antibodies.	Less than (<) 5.6 IU/ml
TG (thyroglobulin).	3 to 40 ng/mL
TSAb (Thyroglobulin antibodies)	< 4 IU/ml
Thyroid-stimulating hormone receptor antibodies (TRAb)	< 1.75 IU/L or <3.3 IU/L
TSI (thyroid-stimulating immunoglobulin).	< 0.55 IU/ml

In pregnant women, thyroid-binding globulin (TBG) production is increased because of estrogen and beta-human chorionic gonadotropin (beta-HCG). More free T4 will be bound to TBG, leading to increased production of T4. TSH levels are normal. Therefore, laboratory values will show normal TSH, normal free T4, and elevated total T4.In addition to pregnancy, elevated TBG levels could also be due to hypothyroidism, liver disease, and pregnancy.

In addition to blood tests, imaging tests like thyroid ultrasound and thyroid scans may be used to assess the size, shape, and structure of the thyroid gland, and to identify any nodules or abnormalities.

While thyroid functions are important in overall growth and development of an individual, human chorionic gonadotropin hormone plays crucial role in early development during pregnancy.

SAQ 1

a) What is cause of primary and secondary hypothyroidism?

b) Which diseases cause primary and secondary hyperthyroidism?

c) Write general symptoms hypothyroidism and hyperthyroidism.

14.4 HUMAN CHORIONIC GONADOTROPIN (HCG)

Human chorionic gonadotropin(hCG) is a hormone produced by trophoblast cells of early embryo, that eventually develop into a part of placenta. Since hCG is produced immediately rising on day 8-10 after conception supporting the production of estrogens and progesterone in the corpus luteum, therefore, it is measured to detect pregnancy and to distinguish between normal and abnormal pregnancies.It is also useful for monitoring the progress after a pregnancy loss. In addition, hCG testing can aid in the diagnosis of certain cancers, such as choriocarcinoma and some extra-uterine malignancies. Due to its role in pregnancy, exogenous administration of hCGis used as a standard practice for *in vitro* fertilization transfer care.

Levels of hCGover 20 IU/L in urine indicate pregnancy test. Absolute values may vary individually during pregnancy; a rising trend is always important (hCG doubles every two to three days at the beginning of pregnancy); a drop may be indicative of a missed abortion. Repeated hCG blood tests are important for diagnosing ectopic pregnancyasthe rise in hCG is usually less steep. If the increase in hCG concentration is less than 60% over 48 hours, it indicates an ectopic pregnancy or death *in utero*. HCG tests are also useful in diagnosing trophoblastic diseases, or as a germinal tumour marker; an elevated hCG level may also be caused by myomas and ovarian cysts.

14.5 SUMMARY

- Hormones are the chemical messengers that participate in wide range of activities ranging from birth, growth and development, metabolism, reproduction, mental health to aging.
- Hormonal imbalances occur when there is too much or too low of a hormone in the system leading to disruption of normal functions and to a range of health issues, making the study of hormones crucial for diagnosis and treatment of various conditions.
- Diabetes, thyroid disorders (hyperthyroidism and hypothyroidism), and conditions like PCOS (Polycystic Ovary Syndrome) are some of the diseases which are due to hormonal imbalance.
- The thyroid gland constitutes part of hypothalamic-pituitary-thyroid axis referred to as a self-regulatory circuit. Thyroid gland secrete thyroxine or tetraiodothyronine (T4) and triiodothyronine (T3) and calcitonin.
- Thyroid hormones are secreted when stimulated by TSH by secreted by the anterior pituitary gland that in turn is stimulated by the thyrotropin releasing hormone (TRH) of hypothalamus.
- Thyroid gland malfunction may manifest as hypothyroidism or hyperthyroidism, which may be of primary (due to abnormality in thyroid gland) or secondary (due to abnormality pituitary) or tertiary (due to hypothalamic abnormality) origin.

- Graves disease is the most common cause of hyperthyroidism and Hashimoto thyroiditis is the most common cause of hypothyroidism in iodine-sufficient areas and is caused by autoimmune-mediated destruction of the thyroid gland.
- Diagnosis of thyroid functions is done based on two types of tests: blood and imaging tests. Blood tests generally include testing for TSH, T3 and T4, and thyroid antibodies. The initial tests of choice to screen for any thyroid abnormality are a TSH and a free thyroxine (free T4) test. These determine whether the abnormality arises centrally from the thyroid gland (primary), peripherally from the pituitary (secondary), or hypothalamus (tertiary).
- In addition to blood tests, imaging tests like thyroid ultrasound and thyroid scans may be used to assess the size, shape, and structure of the thyroid gland, and to identify any nodules or abnormalities.
- Human chorionic gonadotropin(hCG) is a hormone produced by trophoblast cells of early embryo, that eventually develop into a part of placenta.
- It is measured to detect pregnancy and to distinguish between normal and abnormal pregnancies. It is also useful for monitoring the progress after a pregnancy loss.
- In addition, hCG testing can aid in the diagnosis of certain cancers, such as choriocarcinoma and some extra-uterine malignancies. Due to its role in pregnancy, exogenous administration of hCG is used as a standard practice for in vitro fertilization transfer care.

14.6 TERMINAL QUESTIONS

1. Which tests are included in the thyroid function tests? What type of information do these provide about various diseases.
2. Explain clinical significance of human chorionic gonadotropic hormone.
3. Write short notes on Grave's disease and Hashimoto thyroiditis.

14.7 ANSWERS

Self Assessment Questions

1. a) In primary hypothyroidism, decreased production of thyroid hormones by the thyroid gland causes a compensatory increase of TSH. Secondary hypothyroidism is caused by pituitary disorders causing decreased TSH release and decreased T3/T4 levels.

   b) In primary hyperthyroidism, hyperactivity of the thyroid gland can result in excess T3 and T4 production along with the compensatory decrease of TSH. Secondary cause of T3 and T4 production is thyrotroph adenoma.

c) Generalized symptoms associated with hypothyroidism include slow basal metabolic rate that can present as apathy, slowed cognition, skin dryness, alopecia, increased low-density lipoproteins, and increased triglycerides.

Generalized symptoms of hypermetabolism are excessive heat increased contractility and increased cardiac output.

Terminal Questions

1. Thyroid function tests include measurement of TSH, T3 and T4, and thyroid antibodies in blood and imaging of thyroid. Please refer to the section 14.3.2 for more details.

2. It is measured to detect pregnancy and to distinguish between normal and abnormal pregnancies. It is also useful for monitoring the progress after a pregnancy loss. In addition, hCG testing can aid in the diagnosis of certain cancers, such as choriocarcinoma and some extra-uterine malignancies. Due to its role in pregnancy, exogenous administration of hCG is used as a standard practice for in vitro fertilization transfer care.

3. Please refer to the section 14.3.1 for details of these diseases.