

1. **Introduction and literature review**

1.1 Introduction:

The thyroid gland is one of the largest endocrine glands in the body. In healthy people it produces thyroid hormones (TH) which are important at a cellular level, affecting the growth development and rate of function of many other systems in the body (Markovits et al, 2007). It has functions as a stimulus to metabolism and is critical to normal function of the cell. These hormones also have direct effect on most organs including heart, beats faster and harder under influences of thyroid hormone. The thyroid hormone control how quickly the body burns energy, makes proteins, and how sensitive the body should be to other hormones. As well as many functions of the liver, this is the primary organ responsible for the maintenance of the metabolic process (Bartalena et al, 1998)

Overt abnormalities in thyroid function are common affecting 5-10% of individual over life span. Clinical symptoms and signs are often non specific and the diagnosis depends on measurements of thyroid hormones (Growder et al, 2011).

Thyroid disorders are commonly separated into two major categories, hyperthyroidism and hypothyroidism, depending on whether serum thyroid hormone levels (T_4 and T_3) are increased or decreased, respectively (Ruiter, 2002).

The serum creatine kinase enzyme activity in healthy individuals depends on age , race , body mass and physical activity. It has since become an important clinical marker for muscle damage (Growder et al, 2011).

Musculoskeletal disorders often accompany thyroid dysfunction. In addition to the well known observation that these disorders are common in patients with hypothyroidism, they are also observed in patients with thyrotoxicosis. Out of some common causes, hypothyroidism is a common cause of an elevated creatine kinase in serum (Jnkins, 1978).

AST and ALT enzymes are distributed widely in tissues, and normally present in plasma, bile and cerebrospinal fluid (tietz et al, 2001).

1.2 Literature review:

1. 2.1Thyroid gland:

The thyroid is a small butterfly shaped endocrine gland, located in the lower part of the neck, in front of the windpipe which secretes thyroid hormones (Malik et al, 2010).

The thyroid gland secretes thyroxin (T_4) and Triiodothyronine (T_3), both of which modulate energy utilization and heat production and facilitate growth. The gland consists of two lateral lobes joined by an Isthmus. The weight of the adult gland is 10 to 20 g. microscopically; the thyroid is composed of several follicles containing colloid surrounded by a single layer of thyroid epithelium. The follicular cells synthesize thyroglobulin, which is then stored as colloid. Biosynthesis of T_4 and T_3 occurs by iodination of tyrosine molecules in thyroglobulin (Andreoli et al, 2010).

1.2.1.1 Thyroid hormones:-

- Thyroxin (T_4)
- Triiodothyronine (T_3)

More T_4 than T_3 is produced, but T_4 is converted in some peripheral tissues (liver, kidney, and muscles) to the more active T_3 (Kumar et al, 2002).

In plasma more than 99% of all T_4 and T_3 is bound to hormone bound proteins:

- Thyroxine-binding globuline (TBG).
- Thyroid-binding prealbumin (TBPA).
- Albumin.

Only free hormone is available for tissue action, where T_3 binds to specific nuclear receptors within the cell (Kumar et al, 2002).

Synthesis of thyroid hormones:

Dietary iodine is essential for synthesis of thyroid hormones. Iodine, after conversion to iodide in the stomach, is rapidly absorbed from the gastrointestinal tract and distributed in the extracellular fluids. After active transport from the blood stream across the follicular cell basement membrane, iodide is enzymatically oxidized by thyroid peroxidase, which also mediates the iodination of the tyrosine residues in thyroglobulin to form monoiodotyrosine and diiodotyrosine. The iodotyrosine molecules couple to form T_4 or T_3 . Once iodinated, thyroglobulin containing newly formed T_4 and T_3 is stored in the follicles. Secretion of free T_4 and T_3 into the circulation occurs after proteolytic digestion of thyroglobulin, which is stimulated by thyroid-stimulating hormone (TSH). Deiodination of

monoiodotyrosine and diiodotyrosine by iodotyrosine deiodinase releases iodine, which then reenters the thyroid iodine pool (Andreoli et al, 2010).

Control of thyroid hormones:-

Thyroid stimulating hormone (TSH) stimulates the synthesis and release of thyroid hormones from the thyroid gland. Its secretion from the anterior pituitary gland is controlled by thyrotrophin-releasing hormone (TRH) and circulating concentrations of thyroid hormones (Crook, 2007).

Hypothalamic thyrotropin-releasing hormone (TRH) is transported through the hypothalamic-hypophyseal portal system to the thyrotrophs of the anterior pituitary gland. Hypersecretion of TSH results in thyroid enlargement (goiter). Circulating T₃ exerts negative feedback inhibition of TRH and TSH release (Andreoli et al, 2010).

Thyroid hormones function:-

- Control of energy expenditure is the primary function of thyroid hormones.

In addition:

- They are indispensable for growth, development, and sexual maturation in mammals.
- Stimulation of heart rate and heart contraction.
- Stimulation of protein synthesis and carbohydrates metabolism.
- Increase in synthesis of cholesterol and triglyceride.
- Increase in vitamins requirements (Tietz et al, 2001).

1.2.1.2 Thyroid evaluation:

Thyroid gland function and structure can be evaluated by:

- serum thyroid hormone levels,
- imaging of thyroid gland size and architecture,
- measurement of thyroid auto antibodies,
- And thyroid gland biopsy (by fine-needle aspiration (Andreoli et al, 2010).

Thyroid function tests:-

Immune assays for free T₄, free T₃ and TSH are widely available.

1. **TSH measurement:** TSH levels can discriminate between hyperthyroidism, hypothyroidism and euthyroidism (Kumar et al, 2002).

2. **Free T4 and T3 tests:** Attempts to measure only the unbound hormones (Kumar et al, 2002).

3. **TRH test:** Used to confirm diagnosis of secondary hypothyroidism or to diagnose early primary hypothyroidism (Crook, 2007).

Problems in interpretation of thyroid function tests:-

1. Serious acute or chronic illness:

Thyroid function affected by several ways:

- a) Reduced concentration and affinity of binding protein.
- b) Decrease peripheral conversion of T₄ to T₃ with more r T₃.
- c) Reduced hypothalamic pituitary TSH production (Kumar et al, 2002).

2. Pregnancy and oral contraceptives: These lead to greatly increase TBG level (Kumar et al, 2002).

3. Drugs: Many drugs affect thyroid function test by interfering with protein binding (Kumar et al, 2002).

1.2.1.3 Thyroid disorders:

Thyroid disorder is a general term representing several different diseases involving thyroid hormones and the thyroid gland. Thyroid disorders are commonly separated into two major categories, hyperthyroidism and hypothyroidism, depending on whether serum thyroid hormone levels (T_4 and T_3) are increased or decreased, respectively (Ruiter, 2002).

The prevalence and incidence of thyroid disorders is influenced primarily by sex and age. Thyroid disorders are more common in women than men, and in older adults compared with younger age groups (Ruiter, 2002).

I. Goiter:

Enlargement of the thyroid gland is called goiter. Patients with goiter may be euthyroid (simple goiter), hyperthyroid (toxic nodular goiter, or Graves' disease), or hypothyroid (nontoxic goiter, or Hashimoto's thyroiditis). Thyroid enlargement (often focal) also may be due to a thyroid adenoma or carcinoma. In nontoxic goiter, inadequate thyroid hormone synthesis leads to TSH stimulation with resultant enlargement of the thyroid gland. Iodine deficiency (endemic goiter) was once the most common cause of nontoxic goiter; with the use of iodized salt, Dietary goitrogens can cause goiter, and iodine is the most common goitrogen. Other goitrogens include lithium and vegetable products such as thioglucosides found in cabbage.

Thyroid hormone biosynthetic defects can cause goiter associated with hypothyroidism or, with adequate compensation, euthyroidism (Andreoli et al, 2010).

II. Euthyroid sickness syndrome:-

In the euthyroid sick syndrome (or sick euthyroid syndrome"), thyroid tests are abnormal even though the thyroid gland is functioning normally (Ruiter, 2002).

The euthyroid sick syndrome commonly occurs in patients who have a non-thyroid, severe illness such as heart failure, chronic renal failure, liver disease, stress, starvation, surgery, trauma, infections, and autoimmune diseases, as well as in patients using a number of drugs.

Sick euthyroid syndrome may take one of several diagnostic forms as outlined below:

Low T_3 : This is the most commonly encountered abnormality in non-thyroidal illness. TSH and total and free T_4 levels are usually normal.

Low T_3 and low T_4 : In patients who are moderately ill, low T_3 levels are accompanied by low T_4 levels.

Low TSH, low T_3 , and low T_4 : This abnormality occurs in patients with the most severe non-thyroidal illness.

Elevated T_4 : In this condition, the total T_4 level is elevated, TSH level is normal or elevated, and T_3 level is normal or high. It may be seen in primary biliary cirrhosis and acute and chronic active hepatitis, in which TBG synthesis and release are, increased (Ruiter, 2002).

III. Hypothyroidism:-

Hypothyroidism is a clinical syndrome caused by deficiency of thyroid hormones (Andreoli et al, 2010).

Hypothyroidism is classified as primary or secondary.

A. Primary hypothyroidism:

Under activity of the thyroid gland is usually primary, from disease of the thyroid (Kumar et al, 2002).

In primary hypothyroidism the loss of thyroid function results in increased TSH secretion which promotes goiter formation (Ruiter, 2002).

Types of primary hypothyroidism:

1. Atrophic (autoimmune) hypothyroidism:

This is the most common cause of hypothyroidism and is associated with anti-thyroid auto antibody leading to lymphoid infiltration of the gland and eventual atrophy and fibrosis. It is six times more common in females and in the incidence increases with age (Kumar et al, 2002).

2. Hashimotos thyroiditis:

This form of autoimmune thyroiditis, also more common in females and more common in late middle age (Kumar et al, 2002).

Hashimoto's disease, or Hashimoto's thyroiditis, has since been characterized as a form of chronic autoimmune thyroiditis. For unknown reasons, the body initiates an autoimmune reaction, creating antibodies that attack the thyroid gland; T lymphocytes directed against normal antigens on the thyroid membrane probably interact with thyroid cell-membrane antigens, which lead to activation of B lymphocytes to produce antibodies. Thyroid peroxidase antibodies, which lead to cellular changes in the

thyroid gland, Hashimoto's thyroiditis patients may develop a goiter or have thyroid atrophy (Ruiter, 2002).

3. Postpartum thyroiditis:

This usually a transient phenomenon observed following pregnancy and may involve hyperthyroidism, hypothyroidism, or the two sequentially (Kumar et al, 2002)

4. Iodine deficiency Thyroid Enzyme Defects, Thyroid hypoplasia and Goitrogens. In adults, iodine deficiency or excess, and the ingestion of goitrogens may cause hypothyroidism on rare occasions by decreasing thyroid hormone synthesis or release (Ruiter,2002)

5. Dyshormonogenesis:

This rare condition is due to genetic defects in the synthesis of thyroid hormones; patient develops hypothyroidism with goiter. (Kumar et al, 2002).

B. **Secondary hypothyroidism**

May be caused by:

1). Insufficient stimulation of the thyroid from hypothalamic (decreased TRH secretion) or pituitary (decreased TSH secretion) disease.

2). Peripheral resistance to thyroid hormones (Ruiter, 2002).

Clinical Features of Hypothyroidism:-

Children

- Learning disabilities
- Mental retardation
- Short stature
- Delayed bone age
- Delayed puberty

Adults

- Fatigue
- Cold intolerance
- Weakness
- Lethargy
- Weight gain
- Constipation
- Menstrual irregularities
- Coarse, thin hair and hair loss
- Dry, coarse, cold skin
- Hoarse voice

Other abnormalities occur in association with hypothyroidism include:-

- Anemia
- Increase serum aspartate transferase.
- Increase serum creatine kinase levels.
- Hypercholesterolemia.
- Hyponatraemia (Kumar et al,2002).

Laboratory investigation of hypothyroidism:-

A careful history should be taken and examination performed checking for goiter.

- The plasma TSH and total T_4 or fT_4 concentration should be measured.
- Slightly elevated plasma TSH and normal T_4 concentrations suggest compensated hypothyroidism.
- Raised plasma TSH and low fT_4 concentration suggest primary hypothyroidism.
- Low plasma TSH and low fT_4 concentrations may indicate secondary hypothyroidism. A TRH test should be done.

Raised plasma TSH and raised plasma fT_4 concentrations may indicate thyroid hormone resistance (Crook, 2007).

IV. Hyperthyroidism:-

Thyroid over activity or Thyrotoxicosis is common, affecting about 2-5% of all females at some times and with a sex ratio of 5:1 most often between ages 20-40 years. Nearly all cases are caused by intrinsic thyroid disease; a pituitary cause is extremely rare (Kumar et al, 2002).

A. Hyperthyroidism associated with thyroid:

1.Graves disease:-

Graves' disease, the most common cause of thyrotoxicosis, is an autoimmune disease characterized by a variety of circulating antibodies, as well as being associated with other autoimmune diseases such as type one diabetes mellitus, adrenal insufficiency and pernicious anemia (Crook, 2007).

In Graves' disease one or more of the following features are present:

- goiter;

- thyrotoxicosis;
- eye disease.
- thyroid dermopathy, usually presenting as marked skin thickening (Andreoliet al, 2010).

2. Toxic Multinodular Goiter: also termed (Plummer's Disease) and produces symptoms similar to Grave's disease (Ruiter, 2002).

3. Solitary toxic adenoma

4. TSH secreting pituitary tumor

5. Iodine induced hyperthyroidism (Ruiter, 2002).

B. Hyperthyroidism not associated with thyroid:

1. Sub acute thyroiditis: granulomatous thyroiditis is an acute inflammatory disorder of the thyroid gland, probably secondary to viral infection, which resolves completely in 90% of cases (Andreoli et al, 2010).

2. Exogenous Sources of Thyroid Hormone: (Thyrotoxicosis factitia)

Thyrotoxicosis factitia is the term used to describe hyperthyroidism resulting from the ingestion of thyroid hormone. Thyroid hormone is used for the treatment of hypothyroidism and non-toxic goiters, and also has been used for the treatment of non-thyroidal diseases including obesity (most common non-thyroidal use), menstrual irregularities, infertility, and baldness. When used for these conditions, excessive dosing of thyroid hormone can result in hyperthyroidism with many of the classic symptoms (Ruiter, 2002).

Clinical features of hyperthyroidism:

- General: Weakness and fatigue.
- Heat intolerance.
- Nervousness, irritability and insomnia.
- Weight loss or gain (increased appetite).
- Diarrhea, frequent bowel movements.
- Palpitations.
- Pedal edema.
- Amenorrhea/light menses (Ruiters, 2002).

Laboratory investigation of suspected hyperthyroidism:

A careful history should be taken and examination performed, checking for goiter. The plasma TSH, fT_3 concentration should be measured (Crook, 2007)

- The plasma fT_4 and fT_3 concentrations are clearly high and the TSH concentration is suppressed.
- In the face of suppressed plasma TSH, a clearly elevated plasma fT_3 concentration confirms diagnosis of hyperthyroidism.
- Measurement of thyroid antibodies is useful.
- The rare TSH secreting pituitary tumours need pituitary assessment.
- Radio iodine up take studies of the thyroid can be useful to distinguish some of the causes of hyperthyroidism.
- The TRH test is sometimes useful in the diagnosis of unclear cases (Crook, 2007)

Biological effect of thyroid disorders:

The function of the thyroid gland is one of the most important in the human body as it regulates majority of the body's physiological actions. The thyroid produces hormones (T_3 and T_4) that have many actions including metabolism, development, protein synthesis, and the regulation of many other important hormones. Any dysfunction in the thyroid can affect the production of thyroid hormones (T_3 and T_4) which can be linked to various pathologies throughout the body (Mohamedali et al, 2014). Such as:

1. Thyroid hormones have significant effects in synthesis mobilization and metabolism of lipids. Hypothyroidism is associated with significant increase of total and low density lipoproteins (P. Walsh et al, 2005).
2. Un treated hyper and hypo thyroidism have been reported to be common cause of heart failure (Biondi et al, 2012).
3. Thyroid hormones may further alter carbohydrates metabolism. Hyperthyroidism impairs glycemic control in

diabetic subjects, while hypothyroidism may increase susceptibility to hypoglycaemia (Hage et al, 2011).

4. As these hormones affect most of the metabolic pathways in the body, purine metabolism is one of these metabolic pathways can be affected by disturbance in thyroid hormones , that can alter the uric acid level , which may lead to hyperuricemia, that can cause the gout .(bishop et al, 2000).
5. Thyroid dysfunctions are most prevalent in women during their most fertile years (15 - 35), and for a long time thyroid dysfunctions have been linked with poor reproductive health and pregnancy outcomes (Jhon et al, 2010).
6. Thyroid diseases including both hypo- and hyperthyroidism are associated with several types of glomerulonephritis. Changes in the serum levels of thyroid hormone can affect nephrotic syndrome in many ways. Due to proteinuria, there is a loss of many binding proteins including thyroxinebinding globulin (TBG), transthyretin or prealbumin, and albumin, and losses of these proteins lead reduction in serum T4 and total T3 levels(Iglesias et al, 2009).
7. Thyroid hormones alteration can affect the entire metabolism. Most affected organs include liver and heart. So, it alters the liver enzymes like ALP, AST, ALT, GGT and cardiac enzymes like CPK, LDH and AST (Pandey et al, 2013).

1.2.2 Enzymes:-

1.2.2.1 Creatine kinase enzyme: (E.C.2.7.3.2)

CK is an enzyme with a molecular weight of approximately 82,000 that is generally associated with ATP regeneration in contractile or transport systems. Its predominant physiologic function occurs in muscle cells (bishop et al,2010).

CK is widely distributed in tissue, with highest activities found in skeletal muscle, heart muscle, and brain tissue. CK is present in much smaller quantities in other tissue sources, including the bladder, placenta, gastrointestinal tract, thyroid, uterus, kidney, lung, prostate, spleen, liver (bishop et al, 2010).

- CK MM: is the predominant isoenzyme in skeletal and cardiac muscles.
- CK MB: about 35% of total CK activity in cardiac muscles and less than 5% in skeletal muscles.
- CK BB: present in high concentration in brain and smooth muscles (Crook,2007).

Clinical significance of CK activity elevation:-

Ck activity is elevated in many diseases include:

1. Diseases of skeletal muscles: Serum Ck activity is elevated in all types of muscular dysatrophy.
2. Disease of the heart: Serum ck enzyme level changes after myocardial infarction.
3. Disease of central nervous system.
4. Disease of thyroid: Serum Ck activity demonstrates an inverse relationship with thyroid activity. Hypothyroidism patients show elevation in activity and in hyperthyroidism the serum CK activity tends to be at the low end of reference interval. (tietz et al,2001).

The determination of creatine kinase (CK) and creatine kinase MB (CK-MB) plays a major role in the differential diagnosis and in monitoring of myocardial infarction patients (Penitila, 2000).

1.2.2.2. Aminotransferases:-

Aminotransferases are enzymes involved in transfer of 2-aminoacid to a 2-oxacid (Crook, 2007).

Aminotransferases include:-

1-Asparte aminotransferase (AST) (EC 2.6.11):

Also named (glutamate oxaloacetate aminotransferase, GOT)

AST is widely distributed in human tissue. The highest concentrations are found in cardiac tissue, liver, and skeletal muscle, with smaller amounts found in the kidney, pancreas, and erythrocytes (bishop et al, 2010).

The clinical significance of AST elevations is that the elevations occur in patients with myocardial infarction, viral hepatitis, and skeletal muscle disease (Hubbard, 2010).

2-Alanine aminotransferase (ALT) (EC 2.6.1.2):

Also named (glutamate pyruvate aminotransferase, (GPT)

ALT is distributed in many tissues, with comparatively high concentrations in the liver. It is considered the more liver-specific enzyme of the transferases (bishop et al, 2010).

ALT is markedly increased in:

- Shock and hypoxia
- Acute viral or toxic hepatitis(Crook, 2007).

Alanine aminotransferase (ALT) has been proved particularly more useful in the evaluation of hepatic disease because it is found in greater concentration in the liver whereas its concentration in heart, skeletal muscles and kidney is lower ALT is more specific for hepatic disease than AST(Cuccherini et al, 1983).

In chronic hepatocellular injury, ALT is more commonly elevated than AST; however, as fibrosis progresses, ALT activities typically decline, and the ratio of AST to ALT gradually increases, so that by the time cirrhosis is present, AST is often higher than ALT (Williams et al, 1988). One notable exception to the

predominance of serum ALT activity in chronic liver disease is alcoholic liver disease where AST activity is generally higher than ALT levels (Sheth et al, 1998).

1.3 Rationale:

Thyroid gland diseases vary according to the environment, diet, heredity and social background. Iodine deficiency is one of the commonest environmental factors responsible for thyroid diseases, More than one billion persons are at risk of iodine deficiency worldwide and 200 million have goitre. In Sudan, iodine deficiency and endemic goitre and variable thyroid dysfunctions are persistent health problems, with the prevalence of goitre reaching up to 22% in some areas, (with a range of 13% to 87%) (medani at al,2011) .

Many studies have been conducted to establish a relationship of creatine phosphokinase activity levels in thyroid diseases. A majority of patients with hypothyroidism have been shown to have an increased serum CK activity, in hyperthyroidism, the serum CK activity tends to be at the low end of the reference interval. (Jnkns, 1978). AST and ALT activities slightly increased in both hyper and hypothyroidism (Pandey et al, 2013).

In spite of the many studies about thyroid disorders, there was no published study found concerning assessment of serum enzymes activity level in hyper and hypo thyroidism in Sudanese patients. That's why we attempt to do this study.

1 .4 Objectives:-

1.4.1 General objective:-

To assess serum activity level of Creatine kinase (CK), Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) and enzymes in Sudanese patients with hypothyroidism.

1.4.2 Specific objective:-

1. To compare the serum activity levels of CK, AST, and ALT of patients with hypothyroidism and control group.
2. To compare the means of CK, ALT and AST activity levels in hypothyroidism patients who under treatment with whom are not under treatment.
3. To correlate between CK, ALT and AST activity levels in hypothyroidism and T_3 T_4 and TSH levels.