

Chapter one

Introduction and Literature review

1.1 Introduction

The thyroid gland, which is the largest and important endocrine gland in human body, is located on the anterior side of the neck, right below the larynx. It has two lobes and is composed of many thin follicular cells with a type of epithelial tissue origin. These follicles store thyroid hormones in the form of Thyroglobulin molecules until the body requires them. Thyroid gland synthesizes and secretes two major hormones, known as 3, 5, 3'-triiodothyronine (T3) and thyroxine, which can sometimes be referred to as 3, 5, 3', 5'-tetraiodothyronine (T4). These hormones are necessary for mediate metabolism rate. These molecules have also critical roles in early brain development, somatic growth, bone maturation, protein synthesis and regulating production of red blood cell. Hormonal output from the thyroid is mediated by thyroid stimulating hormone (also known as TSH or thyrotropin) secreted by anterior pituitary. The secretion of thyrotropin itself is mediated by thyrotropin-releasing hormone, secreted by the hypothalamus. Other effects of thyroid hormones include involvement in hemoglobin production in adult and maturation of Hb in fetus. Thyroid hormones often have important effect on erythropoiesis. They enhance erythropoiesis through hyper proliferation of immature erythroid progenitors and increase secretion of erythropoietin by inducing erythropoietin gene expression. Thyroid hormones also augment repletion of hypoxia inducible factor1 and then motivate growth of erythroid colonies. These hormones also intensify erythrocyte 2, 3 DPG compactness, which enhances the delivery of oxygen to tissues. With regard to lymphocytes, T_3 is as a precursor substance for normal B cell formation in bone marrow through its mediation of pro-B cell proliferation. The most common disorders of the thyroid gland include hyperthyroidism, hypothyroidism and thyroid nodules, which are generally benign thyroid neoplasm but may change to thyroid cancer (Dorgalaleh *et al.*, 2013). Hypothyroidism is a clinical syndrome resulting from deficiency of thyroid hormones leading to generalized slowing of all metabolic processes. metabolic abnormalities associated with hypothyroidism include, anemia, hyperlipidemia and reversible increase in creatinin (Mitra *et al.*, 2010). Hyperthyroidism can have different causes, the more common of which are

autoimmunity (Graves' disease), toxic adenomas (multinodular goiter or single nodule), or the administration of thyroid hormone.(Ford and carter .,1988) .

Hypothyroidism can cause certain forms of anemia on the one hand or hyper proliferation of immature erythroid progenitors on the other hand. In contrast, anemia is not frequently observed in patients with hyperthyroidism, whereas erythrocytosis is fairly common . As far as white blood cells and thrombocytes are concerned, a slightly depressed total leukocyte count, neutropaenia and thrombocytopenia have been observed in hypothyroid patients. Furthermore, elevated, normal or slightly depressed total leukocyte counts have been found in hyperthyroid patients, with only a relative decrease in the number of neutrophils and a relative increase in the number of eosinophils and mononuclear cells . Nevertheless, hyperplasia of all myeloid cell lines in hyperthyroidism and their hypoplasia in hypothyroidism (Kawa *etal* ., 2010) . Therefore, thyroid disorders can induce different effects on various blood cell lineages (Dorgalaleh *etal*., 2013).

1.2 Literature review

Formation of blood cells occurs at different anatomical sites during the course of development from embryonic to adult life. Production of blood cells commences in the yolk sac of the embryo, but then shifts to the liver, and to a lesser extent to the spleen, so that these organs become the dominant sites of production between the second and the seventh month of gestation. The liver and spleen are then superseded by the bone marrow, which serves as the only important site of blood cell production after birth. An exception is lymphocyte production, which occurs substantially in other organs, in addition to the bone marrow, in adult life. Haemopoietic tissue fills all of the cavities within the bones of the newborn, but with increasing age, become localized in the cavities of upper shafts of the femur and the humerus, the pelvis, spine, skull, and bones of the thorax. The total volume of haemopoietic tissue in adults is 1-2 litres. This tissue is referred to as red marrow because of its macroscopic appearance; the remaining bone marrow in the more peripheral regions of the skeleton contain predominantly fat, and is termed yellow marrow. Yellow marrow also occupies a volume of 1-2 litres, and serves as a reserve space into which haemopoietic tissue can expand in response to an increased demand for blood cell production. Only in pathological situation does significant haemopoietic activity occur in the liver, spleen and other sites during adult life, when it is referred to as extramedullary haemopoiesis (Firkin *et al.*, 1989).

All of the blood cells are the progeny of a single type of cell. hematopoietic stem cell. The processes involved in production of all the various cells of blood from the hematopoietic stem cells are collectively called haemopoiesis.

These processes of haemopoiesis include self renewal stem cell, commitment of most progeny of stem cells to differentiate ultimately into particular cell type, proliferation of progenitor cells, and their differentiation along a pathway leading to a particular kind of mature blood cell (Lee *et al.*, 1999)

1.2.1 Cellular components of the blood:-

Red cells, leucocytes and platelets constitute the essential cellular components of the blood. The rates at which these cells are produced are regulated in healthy individuals to match the rates at which they leave the circulation. The concentration of each cell type is consequently maintained in the blood within well-defined limits, unless the

balance between production and elimination is disturbed by pathological processes (Firkin *et al.*, 1989).

1.2.1.1 Red blood cell:-

Erythropoiesis is the process by which red blood cells (erythrocytes) are produced. It is stimulated by decreased O₂ in circulation, which is detected by the kidneys, which then secrete the hormone erythropoietin. This hormone stimulates proliferation and differentiation of red cell precursors, which activates increased erythropoiesis in the hemopoietic tissues, ultimately producing red blood cells (Sherwood *et al.*, 2005).

Stages of erythropoiesis:-

Proerythroblasts are the earliest, least mature cells in the erythrocyte forming series (erythropoiesis). Proerythroblasts are characterized by their size (about 20 µm), and by having a very dense nuclear structure with a narrow layer of cytoplasm, homogeneous in appearance, with a lighter zone at the center; they stain deep blue after Romanowsky staining.

Basophilic erythroblasts After mitosis, of proerythroblast their daughter cells display similar characteristics except that they have smaller nuclei. Daughter cells are called basophilic erythroblasts. Their nuclei are smaller and the chromatin is more coarsely structured.

Polychromatic erythroblasts The immature cells in which the cytoplasm displays a grayish blue hue, which are still able to divide,

Orthochromatic erythroblasts the cells in which the cytoplasm is already taking on a pink hue, which contain a lot of hemoglobin and are no longer able to divide, The nuclei gradually condense into small black spheres without structural definition that eventually are expelled from the cells.

Reticulocytes enucleated young erythrocytes contain copious ribosomes that precipitate into reticular (“net-like”) structures after special staining (supra vital stain). (Theml *et al.*, 2004)

Mature red blood cell

Mature erythrocytes are unique among the cells of human tissues in that they normally lack nuclei and cytoplasmic structures such as lysosomes, endoplasmic reticulum and mitochondria. they exist in a large blood vessels as biconcave discs , but their shape changes to a parachute-like confirmation in the capillaries, which have a diameter less than that of erythrocytes in biconcave disc form .The membrane of red

cells are elastic , loss of flexibility or elasticity leads to membrane damage and shape change (Firkin *et al*, 1989) .The main function of red blood cells is to carry O₂ to the tissues and to return carbon dioxide from the tissue to the lung(Hoff brand and Moss, 2011).

1.2.1.1.1 Hemoglobin:-

Hemoglobin is a conjugate protein of molecular weight 64000 KD , consisting of two pairs of polypeptide chains to each of which a haem is attached.

Human hemoglobin exists in a number of types , which differ slightly in the structure of their globinmoiety. However, the haem is identical in all types (Firkin *et al*, 1989).

1.2.1.1.1.1 Hemoglobin synthesis:-

Each red cell contains approximately 640 million hemoglobin molecules. Each molecule of normal adult hemoglobin consist of four polypeptide chains, each with its own haem group . Haem synthesis occurs largely in the mitochondria by a series of biochemical reactions commencing with the condensation of glycine and succinyl coenzyme under the action of the key rate limiting enzyme delta aminolaevulinic acid synthase. Pyridoxal phosphate is a coenzyme for this reaction which is stimulated by erythropoietin. Ultimately , protoporphyrin combines with iron in the ferrous state to form haem , each molecule of which combines with a globin chain made on the polyribosome's , a tetramer of four globin chains each with its own haem group in a pocket is formed to make up a hemoglobin molecule (Hoff brand and Moss, 2011).

1.2.1.1.1.2 Normal hemoglobin types:-

Hemoglobin A comprises about 97 percent of the hemoglobin of adult red cells. It consist of two(alpha α) and two (beta β) chains. Small amount of hemoglobin A are detected in the fetus as early as the eighth week of life, during the first few months of post natal life, hemoglobin A almost completely replace hemoglobin F and the adult pattern is fully established by six month.

Hemoglobin A2 is the minor hemoglobin in the adult red cell. it consist of two (Alpha α) and two delta chains . Is present in very small amount at birth and reaches the adult level of 1.5-3.2 percent during the first year of life.

Hemoglobin F is the major respiratory pigment from early intra-uterine life up to term. Is consist of two (gamma δ) and two (Alpha α) chains . At term hemoglobin F accounts for 70-90 percent of the total hemoglobin, it then falls rapidly to 25 percent

at 1 month and 5 percent at 6 months, the adult level of about one percent is not reached in some children until puberty

Hemoglobin Gower 1 and hemoglobin Gower 2 are confined to the embryonic stage of development . They contain epsilon and zeta chains, hemoglobin Gower 1 consist of two zeta and two epsilon chains, hemoglobin Gower 2 consist of two Alpha and two epsilon chains.

Hemoglobin Bart's is also found in small amounts in cord blood if sensitive techniques are used (Firkin *etal.*, 1989).

1.2.1.1.3 Hemoglobin function:-

The red cells in systemic arterial blood carry O₂ from the lungs to the tissues and return in venous blood with CO₂ to the lungs. as the hemoglobin molecule loads and unloads O₂ the individual globin chains in the hemoglobin molecule move on each other, when O₂ is unloaded the beta chains are pulled apart , permitting entry of the metabolite 2,3_diphosphoglycerate resulting in a low affinity of the molecule for O₂ (Hoff brand and Moss, 2011).

1.2.1.1.4 Pathological changes in hemoglobin:-

The normal values of hemoglobin concentration in normal adults,13.5-17.5g/dl for adult male and 11.5-15.5g/dl for adult females (Hoff brand and Moss, 2011).

Anemia is present when hemoglobin level in the blood is below the lower extreme of the normal range for the age and sex of the individual. And increase in hemoglobin concentration seen in polycythaemia (Firkin *etal*, 1989).

Hemoglobin abnormalities:- these result from the following (Hoff brand and Moss, 2011) :-

-Synthesis of an abnormal hemoglobin.

-Reduced rate of synthesis of normal Alfa or beta globin chains.

The clinical syndromes produced by hemoglobin abnormalities:-

Haemolysis (Hb S)

Thalassaemia (reduced globin chain synthesis)

Familial polycythaemia (altered oxygen affinity)

Methaemoglobinaemia (failure of reduction)

1.2.1.1.2 Haematocrit:-

The haematocrite or packed red cell volume (PCV) refers to the proportion of the volume of red cells relative to the total volume of the blood (Firkin *etal*, 1989).

PCV value is determined by :-

- Microhaematocrite procedure:-

High speed centrifugation used to sediment the red cells.

- Electronic automated devices .

Normal values of haematocrite in adult male is 40-50% and adult female 36-48%.

Reduction of hemoglobin like in anemia is usually accompanied by fall in packed cell volume .

1.2.1.1.3 Red cell indices:-

They are the mean cell volume (MCV) , the mean cell hemoglobin (MCH) and the mean cell hemoglobin concentration (MCHC) . The red cell indices are of considerable clinical importance and are widely used in the classification of anemia (Dacie and Lewis ,1996).

MCV:-

The mean volume of red cells was formerly determined by dividing the total volume of red cells (derived from the packed cell volume) by the number of red cells in that particular blood sample.

Automated electronic particle counting device have revolutionized the estimation of the MCV, most device measure the electrical impedance caused by each red cell as it passes through the counting mechanism and the extent of the impedance provides an accurate indication of the volume of each cell.

The MCV provides index of the average size of red cells, which is a guide of considerable importance to the nature of the disorder underlying an abnormality in hemoglobin level . A subnormal MCV is indicative of microcytosis , and an elevated MCV indicative of macrocytosis.

MCH:-

The mean amount of hemoglobin per red cell , is estimated by dividing the total amount of hemoglobin by the number of red cells in the sample of blood .

A subnormal MCH occurs in microcytosis, but is even lower when microcytosis occurs in conjunction with a subnormal concentration of hemoglobin in the red cell , as in thalassaemia minor or iron deficiency .

MCHC:-

The mean concentration of hemoglobin within red cell is derived by dividing the concentration of hemoglobin by the volume of red cells.

A subnormal MCHC is usually indicative of an abnormality where interference with the synthesis of hemoglobin is greater than that of other constituents of the red cells , as in thalassaemia or iron deficiency .

Elevated values reflect dehydration of the erythrocyte and one of the relatively few important clinical causes of this phenomenon is spherocytosis. (Firkin *et al*, 1989).

1.2.1.2 White blood cell :-

White blood cells, or leukocytes , are the cells of the immune system that are involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist, and several types (including monocytes and neutrophils) are phagocytic (LaFleur-Brooks, 2008). All leukocytes are produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. They live for about three to four days in the average human body. Leukocytes are found throughout the body, including the blood and lymphatic system (Maton *et al*,1997).

Leucopoiesis is the process of white blood cell formation ,:-

Myeloblasts are the least mature cells in the granulocyte lineage. Mononuclear, round-to-ovoid cells, they may be distinguished from proerythroblasts by the finer, “grainy” reticular structure of their nuclei and the faintly basophilic cytoplasm. On first impression, they may look like large or even small lymphocytes, but the delicate structure of their nuclei always gives them away as myeloblasts. In some areas, condensed chromatin may start to look like nucleoli. Sporadically, the cytoplasm contains a zurophilic granules.

Promyelocytes are the product of myeloblast division, and usually grow larger than their progenitor cells. During maturation, their nuclei show an increasingly coarse chromatin structure. The nucleus is eccentric , basophilic cytoplasm contains copious large a zurophilic granules.

Myelocytes are the direct product of promyelocyte mitosis and are always clearly smaller than their progenitors. The ovoid nuclei have a banded structure, the cytoplasm is becoming lighter with maturation and in some cases acquiring a pink tinge. A special type of granules, which no longer stain red like the granules in promyelocytes , are evenly distributed in the cytoplasm.

Metamyelocytes (young granulocytes) are the product of the final myelocyte division and show further maturation of the nucleus with an increasing number of stripes and

points of density that give the nuclei a spotted appearance. The nuclei slowly take on a kidney bean shape. Metamyelocytes are unable to divide. From this stage on, only further maturation of the nucleus occurs by contraction,

Band cells (band neutrophils) represent the further development of metamyelocytes. Distinguishing between the different cell types is often difficult. The term “band cell” should be used when all nuclear sections of the nucleus are approximately the same width (the “bands”).(Theml *et al*, 2004).

Neutrophil (polymorph):-

Neutrophil granulocytes are the most abundant type of white blood cells in mammals and form an essential part of the innate immune system. They are formed from stem cells in the bone marrow. They are short-lived and highly motile. Neutrophils may be subdivided into segmented neutrophils and banded neutrophils (or bands). They form part of the polymorph nuclear cell family (PMNs) together with basophiles and eosinophiles (Witko-Sarsat *et al*, 2000). This cell has a characteristic dense nucleus consisting of two and five lobes and pale cytoplasm with an irregular outline containing many fine pink-blue or grey-blue granules (Hoff brand and Moss, 2011) .The normal value of neutrophil is $2.5 \times 10^9/L$ - $7.5 \times 10^9/L$, an increased numbers of neutrophil series in the blood is termed neutrophilia. When the concentration of neutrophils in the blood is below the lower level of normal is termed neutropenia (Firkin *et al*, 1989).

Lymphocytes:-

Are produced everywhere, particularly in the lymph nodes, spleen, bone marrow, and the lymphatic islands of the intestinal mucosa, under the influence of the thymus (T-lymphocytes, about 80%), or the bone marrow(B-lymphocytes, about 20%). A small fraction of the lymphocytes are NK cells (natural killer cells) The cells encountered in circulating blood are mostly “small” lymphocytes with oval or round nuclei 6–9 μm in diameter. The cytoplasm wraps quite closely around the nucleus and is slightly basophilic. Values between 1500 and 4000/ μl reflect normal output of the lymphatic system. Elevated absolute lymphocyte counts(lymphocytosis) (Theml *et al*, 2004).

Monocytes:-

Are a type of white blood cells (leukocytes). They are the largest of all leukocytes. They are part of the innate immune system of vertebrates including all mammals (humans included), birds, reptiles, and fish. They are amoeboid in shape, having clear cytoplasm. Monocytes have bean-shaped nuclei that are unilobar, which makes them one of the types of mononuclear leukocytes (a granulocytes). Monocyte spend only a

short time in the marrow and after circulating for 20-40 hours, leave the blood to enter the tissue where they mature and carry out their principal functions. Arise in blood monocyte count above $0.8 \times 10^9/L$ is termed monocytosis(Hoff brand and Moss, 2011).

Eosinophils:-

Are white blood cells and one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. Along with mast cells, they also control mechanisms associated with allergy and asthma. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood. In normal individuals, eosinophils make up about 1-6% of white blood cells, and are about 12-17 micrometers in size (Young *et al.*, 2006) . The cytoplasmic granules of eosinophil are coarse and deeply staining , there are rarely more than three nuclear lobs. Eosinophilia is an increase in blood eosinophil above $0.4 \times 10^9/L$ I, is most frequently caused by allergic diseases , parasite and skin diseases (Hoff brand and Moss, 2011).

Basophils:-

Are the least common of the granulocytes. Basophils appear in many specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. Basophiles contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. Like eosinophils, basophiles play a role in both parasitic infections and allergies. They have dark cytoplasmic granules which overlie the nucleus, in the tissue they become mast cells. An increase in blood basophiles above $0.1 \times 10^9/L$ is termed basophilia and is uncommon ,the usual cause is a myeloproliferative disorder such as chronic myeloid leukemia or polycythaemia Vera (Hoff brand and Moss, 2011).

1.2.1.3 Platelets:-

Also called "thrombocytes", are blood cells whose function (along with the coagulation factors) is to stop bleeding.

Thrombopoiesis :-

refers to the process of thrombocyte generation.

Stages of thrombopoiesis:-

Megakaryocytes have giant nuclei , which build up by endomitosis. Cytoplasm with granules is pinched off from megakaryocytes to form thrombocytes. The residual naked megakaryocyte nuclei are phagocytosed.

Thrombocyte Only mature thrombocytes occur in blood. About 1–4 μm in size and a nuclear, their light blue stained cytoplasm, with fine reddish blue granules near the center. Young thrombocytes are larger and more “spread out;” older ones look like pyknotic dots (Theml *et al.*, 2004).

The main function of platelets is to contribute to homeostasis: The process of stopping bleeding at the site of interrupted endothelium. They gather at the site and unless the interruption is physically too large, they plug the hole. First, platelets attach to substances outside the interrupted endothelium: adhesion. Second, they change shape, turn on receptors and secrete chemical messengers: activation. Third, they connect to each other through receptor bridges: aggregation (Yip *et al.*, 2005). Low platelet concentration is thrombocytopenia and is due to either decreased production or increased destruction. Elevated platelet concentration is thrombocytosis . A disorder of platelet function is a thrombocytopathy .

1.2.2 The Endocrine system

Refers to the collection of glands of an organism that secrete hormones directly into the circulatory system to be carried toward a distant target organ. The major endocrine glands include the pineal gland, pituitary gland, pancreas, ovaries, testes, thyroid gland, parathyroid gland, hypothalamus, gastrointestinal tract and adrenal glands. The endocrine system is in contrast to the exocrine system, which secretes its hormones using ducts.

1.2.2.1 Pituitary gland:-

The pituitary gland is a pea-sized gland that sits in a protective bony enclosure called the sella turcica. It is composed of three lobes: anterior, intermediate, and posterior. In many animals, these three lobes are distinct. However, in humans, the intermediate lobe is a few cell layers thick and indistinct; as a result, it is often considered part of the anterior pituitary.

The anterior pituitary (adenohypophysis):-

Arises from an invagination of the oral ectoderm and forms Rathke's pouch. This contrasts with the posterior pituitary (neurohypophysis), which originates from neuroectoderm.

Endocrine cells of the anterior pituitary are controlled by regulatory hormones released by parvocellular neurosecretory cells in the hypothalamus.

The posterior pituitary (neurohypophysis), develops as an extension of the hypothalamus. The release of pituitary hormones by both the anterior and posterior lobes is under the control of the hypothalamus (Boron *etal*, 2009).

Functions of pituitary gland:-

The anterior pituitary synthesizes and secretes the following important endocrine hormones:-

-Somatotrophins:

Human growth hormone also referred to as 'growth hormone', and also as somatotropin, is released under the influence of hypothalamic growth hormone-releasing hormone, and is inhibited by hypothalamic somatostatin.

-Thyrotrophins:

Thyroid-stimulating hormone, is released under the influence of hypothalamic thyrotropin-releasing hormone and is inhibited by somatostatin.

-Corticotropins:

Adrenocorticotrophic hormone, and Beta-endorphin are released under the influence of hypothalamic corticotropin-releasing hormone.

The posterior pituitary stores and secretes (not synthesize) the following important endocrine hormones:

- Antidiuretic hormone
- Oxytocin:- is one of the few hormones to create a positive feedback loop. For example, uterine contractions stimulate the release of oxytocin from the posterior pituitary, which, in turn, increases uterine contractions. This positive feedback loop continues throughout labour (Knepel *etal*, 1984).

1.2.2.2 Thyroid gland:-

The thyroid gland is found in the neck, below the thyroid cartilage . The thyroid gland controls how quickly the body uses energy, makes proteins, and controls how sensitive the body is to other hormones. It participates in these processes by producing thyroid hormones, the principal ones being triiodothyronine (T_3) and thyroxine which can sometimes be referred to as tetraiodothyronine (T_4). These hormones regulate the growth and rate of function of many other systems in the body. T_3 and T_4 are synthesized from iodine and tyrosine. The thyroid also

produces calcitonin, which plays a role in calcium homeostasis. Hormonal output from the thyroid is regulated by thyroid-stimulating hormone (TSH) produced by the anterior pituitary, which itself is regulated by thyrotropin-releasing hormone produced by the hypothalamus.

1.2.2.1 Thyroid hormones:-

The thyroid hormones, triiodothyronine (T₃) and thyroxine (T₄), are tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. Iodine is necessary for the production of T₃ and T₄. The major form of thyroid hormone in the blood is thyroxine (T₄), which has a longer half-life than T₃.

1.2.2.2 Synthesis of thyroid hormones:-

Thyroxine (T₄) is synthesized by the follicular cells from free tyrosine and on the tyrosine residues of the protein called thyroglobulin. Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme thyroid peroxidase (Ekholm and Bjorkman, 1997), and linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on thyroglobulin, and on free tyrosine. Upon stimulation by the thyroid-stimulating hormone, the follicular cells reabsorb thyroglobulin and cleave the iodinated tyrosine from thyroglobulin in lysosomes, forming T₄ and T₃ (in T₃, one iodine atom is absent compared to T₄), and releasing them into the blood. Deiodinase enzymes convert T₄ to T₃ (Bianco *et al.*, 2002).

1.2.2.3 Hyperthyroidism:-

Hyperthyroidism, referred to as an overactive thyroid, is a condition in which the thyroid gland produces and secretes excessive amounts of thyroid hormones, triiodothyronine (T₃) and/or thyroxine (T₄) (Dairo *et al.*, 2008).

Causes:- There are several causes of hyperthyroidism. Most often, the entire gland is overproducing thyroid hormone. Less commonly, a single nodule is responsible for the excess hormone secretion, called a "hot" nodule. Thyroiditis (inflammation of the thyroid) can also cause hyperthyroidism. Functional thyroid tissue producing an excess of thyroid hormone occurs in a number of clinical conditions :- grave's disease , toxic thyroid adenoma (Andersson *et al.*, 2010).

Hyper secretion of thyroid stimulating hormone, which in turn is almost always

caused by a pituitary adenoma, accounts for much less than 1 percent of hyperthyroidism cases (Roy and Refetoff, 2009).

Diagnosis:-

The diagnosis of hyperthyroidism is confirmed by blood tests that show a decreased thyroid-stimulating hormone level and elevated T4 and T3 levels.

1.2.2.2.4 Hypothyroidism:-

Often called underactive thyroid, is a common endocrine disorder in which the thyroid gland does not produce enough thyroid hormone.

Hypothyroidism is caused either:- by inadequate function of the gland itself (primary hypothyroidism, due to :- Iodine deficiency , previous thyroidectomy, previous radioiodine treatment,) or by not enough stimulation by thyroid-stimulating hormone (central hypothyroidism , due to :- Lesions compressing the pituitary (pituitary adenoma, meningioma, glioma,), surgery or radiation to the pituitary)

Pathophysiology:-

The hypothalamic–pituitary–thyroid axis plays a key role in maintaining thyroid hormone levels within normal limits. Production of TSH by the anterior pituitary gland is stimulated in turn by thyrotropin-releasing hormone (TRH), released from the hypothalamus. Production of TSH and TRH is decreased by thyroxine by a negative feedback process. Not enough TRH, which is uncommon, can lead to not enough TSH and thereby to not enough thyroid hormone production (Persani, 2012). Pregnancy leads to marked changes in thyroid hormone physiology. The gland is increased in size by 10%, thyroxine production is increased by 50%, and iodine requirements are increased. Many women have normal thyroid function but have immunological evidence of thyroid autoimmunity (as evidenced by auto antibodies) or are iodine deficient, and develop evidence of hypothyroidism before or after giving birth (Stagnaro *etal.*, 2011)

Diagnosis:- An elevated TSH level indicates that the thyroid gland is not producing enough thyroid hormone, and free T4 levels are then often obtained (Garber *etal.*, 2012) . In overt primary hypothyroidism, TSH levels are high and T₄ and T₃ levels are low. TSH usually rises after T₄ and T₃ levels drop(Bona *etal.*, 2013). If the TSH level is normal or low and serum free T₄ levels are low,

this is suggestive of central hypothyroidism (not enough TSH or TRH secretion by the pituitary gland or hypothalamus) (Persani, 2012) . Subclinical hypothyroidism is a milder form of hypothyroidism characterized by an elevated serum TSH level, but with a normal serum free thyroxine level (Fatourechi, 2009).

1.3 Rationale

Thyroid hormones have a crucial role in metabolism and proliferation of blood cells. Thyroid dysfunction induces different effects on blood cells (Dorgalaleh *et al*, 2013). Some studies about these effects was conducted worldwide to determine the complete blood count of patients with hyperthyroidism and hypothyroidism. The aim of this study is to correlate the complete blood count of study group with hyperthyroidism and hypothyroidism. This may give a good chance to follow up patients with hyperthyroidism and hypothyroidism to avoid serious hematological complications.

1.4 Objective

1.4.1 General objective

To determine complete blood count of Sudanese patients with hyperthyroidism and hypothyroidism in Khartoum state.

1.4.2 Specific objectives

- To measure (RBC , HB, HCT,RBC indices , WBC and PLT) of patients with hypothyroidism and hyperthyroidism
- To compare complete blood count of patients and control .
- To verify whether hypothyroidism and hyperthyroidism affect hematopoietic system
- To correlate hypothyroidism and hyperthyroidism with gender .
- To correlate hyperthyroidism and hypothyroidism according to gender with age .

Chapter Two

Materials and Methods

2.1 Study design:

Across sectional study aimed to determine complete blood count in patients with thyroid disease.

2.2 Study population:

100 patients (82 with hyperthyroidism and 18 with hypothyroidism) + 30 healthy(euthyroid) controls.

2.3 Study area:

This study was conducted in Military Hospital in Khartoum state.

2.4 Inclusion criteria:

All patients who were diagnosed with hyperthyroidism or hypothyroidism.

2.5 Exclusion Criteria:

Presence of any diagnosed diseases rather than hyperthyroidism or hypothyroidism

2.6 Ethical considerations:

Ethical clearance was obtained in this study, and the sample was collected after the consent of the participant who were informal about the aim of study and the procedure at the collected.

2.7 Data collection:

Self administrated per-coded questionnaire " personal information includes name, age and sex" was designed to obtain information which was used in the study.

2.8.1 Material:

2.8.1.1 Equipment:

- A. Holder with disposable needle
- B. EDTA vacutainer tube (3 ml)
- C. 70% alcohol "ethanol"
- D. Cotton
- E. Automated hematological analyzer (sysmex KX-N21)

Principle:-

Automated cell counters sample the blood, and quantify, classify, and describe cell populations using both electrical and optical techniques. Electrical analysis involves

passing a dilute solution of the blood through an aperture across which an electrical current is flowing. The passage of cells through the current changes the impedance between the terminals (the Coulter principle). A lytic reagent is added to the blood solution to selectively lyse the red cells (RBCs), leaving only white cells (WBCs), and platelets intact. Then the solution is passed through a second detector. This allows the counts of RBCs, WBCs, and platelets to be obtained. The platelet count is easily separated from the WBC count by the smaller impedance spikes they produce in the detector due to their lower cell volumes. Optical detection may be utilized to gain a differential count of the populations of white cell types. A dilute suspension of cells is passed through a flow cell, which passes cells one at a time through a capillary tube past a laser beam. The reflectance, transmission and scattering of light from each cell is analyzed by sophisticated software giving a numerical representation of the likely overall distribution of cell populations.

2.8.1.2 Reagents:

Commercial close system reagent were provided by sysmex KX-N21 operator and consist of :

- A. Cell pack (stromatolyzer)
- B. Cell cleaner (detergent)

2.8.2 Method

2.8.2.1 Collection of blood sample:

Venous blood was collected under aseptic condition after cleaning the area around the vein with 70% alcohol.3 ml of blood was collected in 3.6 mg EDTA container and it was mixed well before processing.

2.9 Data presentation:

All data was presented in forms at tables.

2.10 Data analysis:

The collected data was analyzed to obtain the mean, Standard deviation and the probability "p.value" between patients and control using SPSS computer program.

Chapter three

Results

Table (3-1) showed age distribution in hyperthyroidism and hypothyroidism patients. 30.5% of hyperthyroidism patients with age ranged between 20-30 years. In hypothyroidism patients the lowest percentage of age ranged between 41-50 years.

Table (3-1) Distribution of test group according to age.

Age group Years	Hyperthyroidism		Hypothyroidism	
	Frequency	Percent%	Frequency	Percent%
20-30	25	30.5	4	22.2
31-40	14	17.1	4	22.2
41-50	21	25.6	2	11.1
51-60	14	17.1	4	22.2
61-70	8	9.8	4	22.2
Total	82	100	18	100

Table (3-2) showed that 87.8% of patients with hyperthyroidism ,and 88.9% of patients with hypothyroidism , were female.

Table (3-2) Distribution of test group according to gender.

Sex	Hyperthyroidism		Hypothyroidism	
	Frequency	Percent%	Frequency	Percent%
Male	10	12.2	2	11.1
Female	72	87.8	16	88.9
Total	82	100	18	100

According to table (3-3) 50% of male patients with age ranged between 61-70 years .and only 8.3% with age ranged between 20-30 years .In female patients the highest percentage of age ranged between 20-30 years and the lowest percentage of age between 61-70 years.

Table (3-3) Distribution of gender of test group according to age.

Age group	Male		Female		
	Year	Frequency	Percent%	Frequency	Percent%
20-30	1	8.3	28	31.8	
31-40	0	0	18	20.5	
41-50	3	25	20	22.7	
51-60	2	16.7	16	18.2	
61-70	6	50	6	6.8	
Total	12	100	88	100	

According to table (3-4) Hct and MCV of hyperthyroidism and hypothyroidism show significant decrease when compared to control group. RBC count showed significant decrease in hypothyroidism patients.

Table (3-4) Comparison of RBC and RBC related indices of patients compared to control

Index		Mean \pmSD	P.value
RBC $\times 10^6$ /ul	Hyperthyroidism	4.6 \pm .5	0.40
	Control	4.7 \pm .4	
	Hypothyroidism	4.4 \pm .4	0.01
Hb g/dl	Hyperthyroidism	12.5 \pm 1.5	0.08
	Control	13.4 \pm 4	
	Hypothyroidism	12 \pm 1.2	0.21
Hct %	Hyperthyroidism	37.5 \pm 4	0.00
	Control	40 \pm 3.7	
	Hypothyroidism	36.7 \pm 3.5	0.00
MCV fl	Hyperthyroidism	80.3 \pm 5.3	0.00
	Control	88 \pm 4.6	
	Hypothyroidism	83.2 \pm 5.4	0.00
MCH pg	Hyperthyroidism	26.9 \pm 2.4	0.03
	Control	28 \pm 2.4	
	Hypothyroidism	27.6 \pm 2.1	0.54
MCHC g/dl	Hyperthyroidism	33.3 \pm 1.2	0.00
	Control	32.5 \pm 1.5	
	Hypothyroidism	33.1 \pm .8	0.09

According to table (3-5) there was no significant differences were noted for total and differential leukocyte count between euthyroid, hyperthyroidism and hypothyroidism group. Plt of patients with hyperthyroidism showed significant decrease when compared to control group.

Table (3-5) Comparison of total, differential leukocyte count and platelete count of patients compared to control.

Indx		Mean±SD	P.value
WBC $\times 10^3/\text{ul}$	Hyperthyroidism	5.5 ±1.3	0.70
	Control	5.6 ±1.5	
	Hypothyroidism	6 ±1.9	0.49
Neutrophil $\times 10^3/\text{ul}$	Hyperthyroidism	2.9±1.1	0.84
	Control	3± 1.2	
	Hypothyroidism	3.3± 1.3	0.41
Lymphocyte $\times 10^3/\text{ul}$	Hyperthyroidism	1.98 ± .5	0.74
	Control	1.94 ± .6	
	Hypothyroidism	2 ±.7	0.51
Monocyte $\times 10^3/\text{ul}$	Hyperthyroidism	.45 ± 1.6	0.71
	Control	.47 ± .15	
	Hypothyroidism	.41 ± .19	0.29
Eosinophil $\times 10^3/\text{ul}$	Hyperthyroidism	.15 ± .09	0.54
	Control	.16 ± .07	
	Hypothyroidism	.18 ± .08	0.34
PLT $\times 10^3/\text{ul}$	Hyperthyroidism	252 ± 58	0.01
	Control	284 ± 68	
	Hypothyroidism	272 ± 79	0.58

Chapter four

Discussion, Conclusion and Recommendation

4.1 Discussion

This is study was carried out in Khartoum state in Military Hospital during the period from February 2014 to April 2014 . One hundred patients were selected 82 with hyperthyroidism and 18 with hypothyroidism , in addition to 30 healthy persons were selected as a control group. The study group selected with age range between 20 and 70 years.

number of males aged between 61 and 70 years , And females aged between 20 and 30 years was higher compared to other age group . The percent of female (88%) is higher than male(12%) in both (hyperthyroidism and hypothyroidism). The results revealed that RBC was significantly decreased in patients with hypothyroidism when compared to control group. This study is similar to a study by(Abdollah Jafarzadeh *et al.*,2010) in Iran, Who found that In hypothyroid group the mean counts of RBC was significantly lower in comparison with euthyroid group ($P<0.05$). The presence of receptor for thyroid hormones has been demonstrated on hematopoietic progenitor cells. It has been also reported that, directly or indirectly, thyroid hormones stimulate growth of erythroid colonies through erythropoietin. It has also been suggested that decreased erythropoietin levels may account for anemia in hypothyroidism (Abdollah Jafarzadeh *et al.*,2010). Hct and MCV showed significant decrease in both hyperthyroidism and hypothyroidism in comparison with control group. this study is similar to study by (Abdollah Jafarzadeh *et al.*, 2010) Some RBC-related indices, such as hematocrit, was also significantly lower in hypothyroid women in comparison to euthyroid group. and In the hyperthyroid group, the mean level of MCV was significantly lower in comparison with the euthyroid group ($P<0.05$). and to study by (Ford and Carter, 1988) in New Zealand. red blood cell indices were compared in patients with hypothyroidism and hyperthyroidism and revealed that MCV in these two groups of patients in comparison to euthyroid individuals have statistically significant difference. ineffective erythropoiesis may occur. some thyrotoxic patients show reduced iron utilization and a reduced mean corpuscular volume is frequently observed. It has been

suggested that these manifestations of ineffective erythropoiesis are most likely to be found in those patients with very severe or long-standing thyrotoxicosis .The present study revealed significant decrease in platelet count in hyperthyroid patients, when compared to control group (P value < 0.05). This study was similar to study by (Ford and Carter, 1988) who reported that, a shortened platelet survival time and an increase in megakaryocytes in bone marrow have been reported in hyperthyroid subjects even when platelet count were normal. uncommon but well recognized association between hyperthyroidism and immune thrombocytopenia. And not similar to a study by (Abdollah Jafarzadeh *et al.*, 2010) they Report no significant difference was found between hyperthyroid and euthyroid groups regarding the mean counts platelets. The exact mechanism for the thrombocytopenia has not been established; however, platelets from such patients show elevated levels of bound immunoglobulin's. It has been pointed out that platelet-bound IgG may not be specific for any platelet antigen, but rather may exist as part of an antigen-antibody complex bound to the Fc receptor of the cell (Ford and Carter, 1988). No significant differences were noted for total and differential leukocyte counts . This was similar to study by (Abdollah Jafarzadeh *et al.*,2010) who concluded no significant differences were noted for total and differential leukocyte counts between euthyroid, hypothyroid and hyperthyroid group .and to study by (Dorgalaleh *et al.*, 2013) in Iran who found that WBC counts in both patient groups compared with the control group did not show any significant differences. In hypothyroid patients, the thyroid hormones T3 and T4 were found at lower concentrations and these lower concentrations of T3 and T4 appears to be sufficient for leukocyte differentiation. In other words, hypothyroid subjects produce thyroid hormones at lower levels but it can be argued that these concentration are sufficient for normal leukocytes differentiation. (Abdollah Jafarzadeh *et al.*,2010).

4.2 Conclusion:

Hyper and Hypothyroidism in Sudanese patients have different effects on complete blood count.

- number of males aged between 61 and 70 years , And females aged between 20 and 30 years was higher compared to other age group
- Large percent (88%) of female in both (hyper and hypothyroidism).
- RBC, Hct and MCV decrease significantly in hypothyroidism patients when compared to control group.
- Hct, MCV, MCH , and PLT decrease significantly in hyperthyroidism patients when compared to control group.
- No significant differences were noted for total and differential leukocyte counts between euthyroid , hyperthyroidism and hypothyroidism group.

4.3 Recommendations:

TSH measurement is recommended in women of childbearing age before pregnancy or during the first trimester.

All patients with thyroid disorder should be periodically evaluated for probable hematological changes by performing complete blood count.

References

Abdollah Jafarzadeh , Masoud Poorgholami , Nazanin Izadi Maryam Nemati, Mohammad Rezayati (2010) , Immunological and hematological changes in patients with hyperthyroidism or hypothyroidism , Clin Invest Med.33,(5).

Andersson, Maria; Zimmermann, Michael B. (2010). Influence of Iodine Deficiency and Excess on Thyroid Function Tests **28**. pp. 45–69.

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002). "Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases". *Endocr Rev* **23** (1): 38–89

Bona, G; Prodam, F; Monzani, A (2013). "Subclinical hypothyroidism in children: natural history and when to treat". *Journal of Clinical Research in Pediatric Endocrinology (Review)*. 5 Suppl 1 (4): 23–8.

Boron, Walter F.; Boulpaep, Emile L. (2009). *Medical Physiology* (2nd ed.). Philadelphia: Saunders Elsevier. pp. 1016–1017

Dacie and Lewis (1996) *Practical Haematology* 8th edition ,Churchill Livingstone.

Dario M. Torre, Geoffrey C. Lamb, Jerome Van Ruiswyk: Kochar's Clinical Medicine for Students. pp.402-403: Lippincott Williams & Wilkins; Fifth edition (2008).

Dorgalaleh , Mahmoodi ,Varmaghani , Saeedi Kia , Alizadeh , Tabibian Sh , Bamedi , Momeni , Abbasian , Kashani Khatib Z,(2013), Effect of Thyroid Dysfunctions on Blood Cell Count and Red Blood Cell Indice . Iranian Journal of Pediatric Hematology Oncology.vol3.No2.

Ekholm R, Bjorkman U (1997). "Glutathione peroxidase degrades intracellular hydrogen peroxide and thereby inhibits intracellular protein iodination in thyroid epithelium". *Endocrinology* **138** (7): 2871–2878

Erdogan Mehme, Kosenli Aybike, Sencer Ganidagli and Kulaksizoglu Mustafa (.2012). Characteristics of anemia in subclinical and overt hypothyroid patients . *Endocrine Journal*.

Fatourechi, V. (2009). "Subclinical Hypothyroidism: An Update for Primary Care Physicians". *Mayo Clinic Proceedings (Review)* **84** (1): 65–71.

Ford and carter (1988) The haematology of hyperthyroidism: abnormalities of erythrocytes, leucocytes, thrombocytes and haemostasis, *Postgraduate Medical Journal*.64,735-742.

Firkin Frank , colin Chesterman , penington and rush ,(1989),de gruchy's *Clinical Haematology in Medical Practice* , Blackwell Science Ltd.

Garber, JR; Cobin, RH; Gharib, H; Hennessey, JV; Klein, I; Mechanick, JI; Pessah-Pollack, R; Singer, PA; Woeber, (December 2012). KA for the American Association of Clinical Endocrinologists and the American Thyroid Association Taskforce on Hypothyroidism in Adults "Clinical Practice Guidelines for Hypothyroidism in Adults". *Thyroid* **22** (12): 1200–1235

Harald Thengl, Heinz Diem, Torsten Haferlach,. (2004). *Color Atlas of Hematology Practical Microscopic and Clinical Diagnosis*. Thieme Stuttgart · New York

Hertz, Barbara, Schuller, Kristin, Saul Hertz, MD (2010) A Pioneer in the Use of Radioactive Iodine, Endocrine Practice

Hoffbrand and Moss ,(2011) ,*Essential Haematology* , 6th edition , Blackwell

Kawa, Katarzyna Grymuła, Edyta Paczkowska, Magdalena Bas'kiewicz-Masiuk, Elzbieta Dałkowska, Monika Koziołek, Maciej Tarnowski, Patrycja Kłos, Violetta Dziedziejko, Magdalena Kucia, Anhelli Syrenicz and Bogusław Machalin'ski .(2010) Clinical relevance of thyroid dysfunction in human haematopoiesis: biochemical and molecular studies. *European Journal of Endocrinology*.162 ,295-305.

Knepel W, Homolka L, Vlaskovska M, Nutto D. (1984). Stimulation of adrenocorticotropin/beta-endorphin release by synthetic ovine corticotropin-releasing factor in vitro. Enhancement by various vasopressin analogs. *Neuroendocrinology*. 38(5):344-50

LaFleur-Brooks, M. (2008). *Exploring Medical Language: A Student-Directed Approach* (7th ed.). St. Louis, Missouri, USA: Mosby Elsevier. p. 398.

Lee G-R, John foerster , john lukens, (1999) *wintrobe's clinical hematology volume 1* , williams and willkins.

Maton., Hopkins, McLaughlin, Johnson, Warner, LaHart, & Wright, Deep V. Kulkarni (1997). *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall.

Mitra Kazemi-Jahromi ,ali shahrirari-Ahmadia ,seyed Hosein samedanifard, Soraya Doostmohamedian. Esmat Abdolhapoor , Seyed Farshad Allameh samedanifard (2010). The association between hypothyroidism and anaemia: a clinical study .*international journal of haematology oncology and stem cell research (IJHOSR)*.

Persani, L (September 2012). "Clinical review: Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges". *The Journal of Clinical endocrinology and Metabolism (Review)* **97** (9): 3068–78.

Roy E Weiss and Samuel Refetoff (2011). Thyrotropin (TSH)-secreting pituitary adenomas Last literature review version 19.1

Sherwood, L, Klansman, H, Yancey, P (2005): Animal Physiology, Brooks/Cole, Cengage Learning.

Stagnaro-Green, A; Abalovich, M; Alexander, E; Azizi, F; Mestman, J; Negro, R; Nixon, A; Pearce, EN; Soldin, OP; Sullivan, S; Wiersinga, W; American Thyroid Association Taskforce on Thyroid Disease During Pregnancy and Postpartum (Oct 2011). "Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum". *Thyroid* **21** (10): 1081–125.

Witko-Sarsat, Rieu , Descamps-Latscha , Lesavre , Halbwachs-Mecarelli (2000). Neutrophils: molecules, functions and pathophysiological aspects.

Yip J, Shen Y, Berndt MC, Andrews RK (2005). "Primary platelet adhesion receptors". *IUBMB Life (International Union of Biochemistry and Molecular Biology: Life)* **57** (2): 103–8

Young, Barbara; Lowe, Joseph O'Connell; Stevens, Alan; Heath, John . (2006). Wheater's Functional Histology (5 ed.). Elsevier Limited.

Normal Values

Parameter	Males	Females	Males and Females
Hemoglobin g/dl	13.5 - 17.5	11.5 – 15.5	
Red cell $\times 10^{12}/L$	4.5 – 6.5	3.9 – 5.6	
PCV%	40 - 52	36 - 48	
MCV fL			80 - 95
MCH pg			27 - 34
MCHC g/dl			20 - 35
WBCs $\times 10^9/L$			4 - 11
Neutrophils $\times 10^9/L$			2.5 – 7.5
Lymphocytes $\times 10^9/L$			1.5 – 3.5
Monocytes $\times 10^9/L$			0.2 – 0.8
Eosinophils $\times 10^9/L$			0.04 – 0.44
Basophils $\times 10^9/L$			0.01 – 0.1
Platelets $\times 10^9/L$			150 - 400

(Hoff brand and Moss, 2011)

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Serial number.....

Name.....

Sex.....

Age.....

Tests:-

TFT :-

TSH.....uU/ml

T4..... ug/dl

T3.....ng/ml

CBC :-

HB..... g/dl

RBC..... $\times 10^{12}/L$

Hct.....%

MCV.....fL

MCH.....pg

MCHC.....g/dl

WBCs..... $\times 10^9/L$

Neutrophils..... $\times 10^9/L$

Lymphocysts..... $\times 10^9/L$

Monocysts..... $\times 10^9/L$

Eosinophils..... $\times 10^9/L$

Basophils..... $\times 10^9/L$

PLT..... $\times 10^9/L$

