HAEMOPARAMETERS IN FEMALE PATIENTS WITH THYROID GLAND DISORDER AT MOI TEACHING AND REFERRAL HOSPITAL

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DECLARATION

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DEDICATION

I dedicate this research to God and my family, Jimmy, Ronny, Salma, and Trevor for their support, love, endurance and understanding, which contributed immensely to completion of this research.

ABSTRACT

Thyroid gland disorders may lead to excessive or deficiency in release of thyroid hormones. The disorder commonly occurs in women and may affect various haemoparameters and present with unclear signs and symptoms not often associated with thyroid disorders. This study investigated the effects of thyroid disorder on haemoparameters (haemoglobin levels, plasma electrolytes, protein metabolites) in female patients at the Moi Teaching and Referral Hospital. This was a cross-sectional study. Fischer formula was used to calculate a sample size of 174 female participants with thyroid disorder and a similar size without thyroid disorder. The inclusion criteria consisted of females aged 15-49 years confirmed thyroid disease and females of corresponding age group without thyroid disease to serve as controls (euthyroid). The exclusion criteria consisted of females outside the stipulated age bracket, pregnant females and those with chronic illness. Venous blood was collected into EDTA vacutainers. Plasma was separated by centrifugation at 3000 rpm and analyzed for urea and creatinine using the Cobas Integra analyzer. Electrolyte levels were determined using the Ion Selective Electrode Analyzer, haemoglobin levels was measured using the Haematology Analyzer, thyroid hormone levels was assessed using the ELISA kit. Data was analyzed using SPSS version 20 and Kruskal Wallis non-parametric statistic, $p<0.05$ was considered significant. Patients with thyroid disorder exhibited elevated levels of plasma urea and creatinine, with a Spearman correlation coefficient of ($r= -0.518, -0.564$) in hyperthyroid and ($r= 0.405, 0.419$) in hypothyroid participants compared to euthyroid participants at ($r= 0.165, 0.037$). The levels of sodium, potassium and chloride ions remained insignificant in all the groups. Low haemoglobin level was observed in the group with hypothyroidism with a Spearman's correlation coefficient of ($r= -0.495$). Haemoglobin levels remained within the normal reference range in the hyperthyroid participants. The findings suggest an increase in plasma urea and creatinine levels in the hyperthyroid and hypothyroid female participants and a decrease in haemoglobin levels in females with hypothyroid disease. Plasma electrolytes remained within the normal reference range. Unclear clinical manifestations are associated with thyroid gland disorder. Screening for thyroid disorder and evaluation of haemoparameters should be advocated for as part of improving the disease management.

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LIST OF ABBREVIATIONS

AACE	American association of clinical endocrinologists
Ag-Ab	Antigen-antibody
ATG-Ab	Anti-thyroglobulin antibodies
ATM-Ab	Anti-microsomal antibodies
ATP	Adenosine triphosphate
AMPATH	Academic model for providing access to health care
cAmp	cyclic adenosine monophosphate
Cl^-	Chloride ion
DALYs	Disability adjusted life years
DIT	Diiodotyrosine
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
EPO	Erythropoietin
FT_3	free triiodothyronine
FT_4	free thyroxine or free tetraiodothyronine
GFR	Glomerular filtration rate
Hb	Haemoglobin
hCG	Human chorionic gonadotrophin
ICCIDD	International council for the control of iodine deficiency disorders
IDD	Iodine deficiency disorders
Ig	Immunoglobulin
IREC	Institutional research and ethics committee
IQR	Inter quartile range
K^+	Potassium ion

MIT	Mono-iodotyrosine
Mmol/L	Millimole per litre
MTRH	Moi teaching and referral hospital
mIU/L	Milli international units per liter
Na^+	Sodium ion
NEMDI	National endocrine and metabolic diseases information service
ng/dL	Nanograms per deciliter
NIS	Sodium-iodide symporter
RBF	Renal blood flow
RPF	Renal plasma flow
RIA	Radio-immuno assay
Rpm	Revolutions per minute
rT_3	Reverse triiodothyronine
SPSS	Statistical package for social sciences
Pg/ml	Picograms per millilitre
T_3	Triiodothyronine
T_4	Thyroxine or tetraiodothyronine
TBG	Thyroid binding globulin
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TRAb	Thyroid stimulating hormone receptor antibodies
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
TSHr	Thyroid stimulating hormone receptor
TSI	Thyroid-stimulating immunoglobulin

WHO	World health organization
µL	Microlitre
2, 3 BPG	2, 3-bisphosphoglycerate

DEFINITION OF TERMS

Erythropoiesis- is the formation of red blood cells (erythrocytes) production, occurring majorly in the bone marrow. Erythropoiesis is initiated by a decrease in O_2 concentration in circulation, detected by the kidneys, which then secrete the hormone erythropoietin, which initiates the red blood cell production.

Infertility- is the inability to conceive after 1 year of regular inhibited intercourse

pH- is a measure of the activity of solution and hydrogen ions. It is the concentration of H^+ and ranges from 0-14. A pH of 7 is said to be neutral, a pH less than 7 is acidic and a pH greater than 7 is basic.

Vasodilation- is the increase in the internal diameter of blood vessels that is caused by relaxation of smooth muscle within the wall of the vessels causing an increase in blood flow.

OPERATIONAL DEFINITIONS

Anaemia- occurs when the concentration of haemoglobin falls below the normal (Hb level 12-18g/dl in non-pregnant women [WHO/CDC 2008]) depending on the person's age, gender and environment, resulting in reduction in the oxygen carrying capacity of blood.

Euthyroid- the physiological state characterized by normal plasma levels of thyroid hormone and normal functioning of the thyroid gland.

Goite- thyroid enlargement caused by compensatory hyperplasia and hypertrophy of the follicular epithelium in response to thyroid hormone deficiency.

Hyperthyroidism- overproduction of thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) caused by hyperfunction of the thyroid gland. The condition is characterized by increased basal metabolism.

Hypothyroidism- a condition caused by any structural or functional derangement that interferes with the production of adequate levels of thyroid hormone.

Screening- the application of a test or tests to detect a potential disease or condition in a person who has no known signs or symptoms of that condition at the time the test is conducted.

Subclinical Hypothyroidism- biochemical evidence of thyroid hormone deficiency in patients who have few or no apparent clinical features of hypothyroidism.

Subclinical Hyperthyroidism- biochemical evidence of thyroid hormone excess in patients who have few or no apparent clinical feature of hyperthyroidism.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The metabolism of all mammalian tissues is regulated by the thyroid hormone produced by the thyroid gland. Overactivity or underactivity of the gland is associated with health problems whose pathophysiology involves multiple body systems (Mullan, 2010).

The thyroid gland synthesizes two hormones L-thyroxine (T_4) and triiodothyronine (T_3) of which T_3 acts at the cellular level. The syntheses of these hormones require dietary iodine, a chemical element present in tiny amounts in the body also known as trace elements. Iodine is trapped by an enzyme dependent system within the gland and is oxidized and incorporated into the glycoprotein thyroglobulin to form mono and diiodotyrosine and then thyroxine and triiodothyronine(HowlettandLevy,2009). The physiological actions of thyroid hormone are categorized as growth, development and regulation of metabolic processes in the body. Thyroid hormone plays a major role in the growth and development of the brain and central nervous system in humans from the 15^{th} week of gestation to 3 years of age. If iodine deficiency exists during this period, the result is thyroid hormone deficiency. The consequences are diminished mental capabilities (Benoist, Anderson and Egil, 2004).

The other physiological role of thyroid hormone is to control several metabolic processes in the body. These include carbohydrate, fat, protein, vitamin and mineral metabolism. Thyroid hormone increases energy production, lipolysis and regulates gluconeogenesis described as the process by which glucose is formed from a non-carbohydrate source such as protein and glycolysis which describes a set of reactions

in which one glucose molecule is oxidized to form two molecules of pyruvic acid and two molecules of adenosine triphosphate (Benoist *et al*., 2004).

Physiological effects of thyroid hormone in the cardiovascular system are to increase the heart rate and cardiac output in order to increase the oxygen needs of the body. In the skeletal system, it increases the bone marrow turnover. In the gastrointestinal tract, it increases gut motility. Thyroid hormone also increases red blood cell formation and facilitates oxygen release to tissues (Howlett and Levy, 2009).

Most thyroid hormone actions (metabolic and developmental) are mediated via nuclear receptors through gene expression regulation. Thyroid hormone receptors are found in most body tissues. T_3 is biologically much more active than T_4 (Skugor and Fleseriu, 2009).

The principal diseases of the thyroid gland include conditions associated with excessive release of thyroid hormone (hyperthyroidism) and those associated with thyroid hormone deficiency (hypothyroidism), thyroiditis, goiter (diffuse or nodular) and mass lesions of the thyroid gland (Kumar, Abul and Nelsonn, 2008). Thyroid diseases frequently seen in Africa include hypothyroidism, thyrotoxicosis, thyroid malignancies and iodine deficiency disorders (Ogbera and Kuku, 2011).

Marc, Stone and Wallace (2003) hyperthyroidism is a hypermetabolic state that describes increased metabolism in the body in which the rate of energy production rises above the normal and results from excess production of T_4 and T_3. Its major clinical manifestations are nervousness, anxiety, rapid pulse, tremors, and muscle weakness, weight loss despite increased appetite and often thyroid gland enlargement known as toxic goiter.

The most common cause of hyperthyroidism is Graves' disease, an autoimmune disease characterized by the production of antibodies that activate the thyroid

stimulating hormone receptor (TSHr) resulting in stimulation of T_4 and T_3 production and enlargement of the thyroid. Other causes of hyperthyroidism are a multinodular goiter, solitary thyroid adenoma, thyroiditis, iodide or drug-induced hyperthyroidism and rarely a thyroid stimulating hormone (TSH) secreting pituitary tumour.

The diagnosis of hyperthyroidism is based on the findings of a high plasma free T_4 level and low plasma TSH concentration. Occasionally, people with hyperthyroidism have a normal plasma free T_4 and high plasma free T_3 concentration (Marc *et al*., 2003).

Hypothyroidism is caused by structural or functional derangement that interferes with the production of adequate levels of thyroid hormone resulting in low thyroid hormone levels. This disorder is classified into primary and secondary categories depending on whether the hypothyroidism arises from an abnormality in the thyroid or occurs because of pituitary disease and rarely hypothalamic failure as a cause of tertiary hypothyroidism (Kumar *et al*., 2008).

Primary hypothyroidism accounts for the majority of cases of hypothyroidism the cause of which include congenital hypothyroidism due to thyroid agenesis, which describes the complete absence of thyroid parenchyma. The gland may be greatly reduced in size, described as hypoplasia. Surgical or radiation of the thyroid parenchyma involving a large resection of the gland for the treatment of hyperthyroidism can lead to hypothyroidism. Available reports show that autoimmune hypothyroidism is the most common cause of hypothyroidism in iodine sufficient areas of the world (Kumar *et al*., 2008).

Autoimmune hypothyroidism is described as a type 2 hypersensitivity reaction, characterized by cells and molecules of the body's own immune system attacking the

thyroid gland resulting in decreased synthesis and secretion of thyroid hormone (Nilsson, 2001).

There are two main types of autoimmune hypothyroidism, Hashimoto's thyroiditis and atrophic thyroiditis. Hashimoto's thyroiditis is the inflammation of the thyroid gland commonly seen in middle-aged women. In this condition, autoantibodies are directed specifically against thyroperoxidase enzymes that catalyze iodide oxidation, iodine transport into the follicle lumen and binding of iodine to tyrosine residues and against thyroglobulin, a glycoprotein containing the amino acid tyrosine from which thyroid hormone is formed (Moore and Dalley, 2006).

Atrophic thyroiditis is a manifestation of the severe stage of hypothyroidism presenting with severe symptoms such as myxoedema which is the accumulation of hydrophilic muco-polysaccarides in the ground substance of subcutaneous tissues (dermis) leading to thickening of the skin (Kumar and Clark, 2003).

Secondary hypothyroidism is caused by thyroid stimulating hormone deficiency and it can result from any of the causes of hypopituitarism a disorder that describes the decreased secretion of one or more of the eight hormones produced by the pituitary gland. The frequent cause being a tumour of the pituitary and trauma. Tertiary hypothyroidism is caused by thyrotropin releasing hormone (TRH) deficiency. Causes include disorders that damage the hypothalamus or interfere with hypothalamic portal blood flow preventing delivery of TRH to the pituitary. The cause of this pathology can result from tumours, trauma and radiation therapy (Kumar *et al*., 2008).

Murgod and Gladys (2012) reported that hypothyroidism is six times more frequent in women than in men, the higher prevalence of thyroid disease in women is probably due to estrogen involvement in the pathophysiology of thyroid dysfunction. Estrogen

(estradiol) has an antagonistic effect on the hormones T_3 and T_4. This is because estradiol competes with T_3 and T_4 for binding sites on the receptor proteins.

There has been an increase in the number of reports that indicate that mild hypothyroidism in early pregnancy increases foetal wastage and impairs the Intelligence Quotient (IQ) of the offspring (Pop *et al*., 1999). The National Academy of Clinical Biochemistry [NACB] (2002) noted mounting evidence to suggest that patients with a persistent TSH abnormality may be exposed to greater risk of severity and complications if left untreated. Such studies support early thyroid function screening especially women in their childbearing years.

It has been suggested that various genetic factors such as the Fas gene found on chromosome 10 encodes one of several proteins important to apoptosis that describes the normal process through which cells die. The Fas gene plays a role in the pathogenesis of autoimmune thyroid disease. Women are more susceptible to the gene activity relative to men and hence are more prone to autoimmune thyroid disease (Turner and Wass, 2004).

Hashimoto's thyroiditis an autoimmune disease results from Fas mediated thyrocyte destruction. Subsequently women who possess this gene are more likely to suffer from thyroid disorders compared to women who lack the gene. Wider (2005) suggested that the susceptibility of occurrence of thyroid gland disease in females than males was due to a genetic link based on their gender than solely based on the effect of estrogen.

Santin and Tanya (2011) noted that thyroid diseases are more prevalent in women especially between puberty and menopause. Fauci and Kasper (2008) pointed out that maternal hypothyroidism occurs in 2–3% of women of childbearing age and is associated with increased risk of developmental delay in the offspring. TSH screening

for hypothyroidism in early pregnancy and in women who are planning pregnancy should be considered, particularly if they have a goiter or strong family history of autoimmune thyroid disease. Women with a precarious iodine intake (<50 g/d) are most at risk of developing a goiter during pregnancy and iodine supplementation should be considered to prevent maternal and foetal hypothyroidism and the development of neonatal goiter. Bowen (2012) noted an increased demand for iodine during pregnancy. The World Health Organization [WHO], (2001) recommends increasing iodine intake from the standard 100 to 150 ug/day to at least 200 ug/day during pregnancy.

Hegazi and Ahmed (2012) recognized unusual manifestations of hyperthyroidism as seen in Graves' disease that are related to various body systems such as the haematopoietic system and the renal system. Similarly, patients with other forms of hyperthyroidism have also displayed atypical manifestations that are often unnoticed and may result in delayed diagnosis and / or misdiagnosis resulting in challenges in the management of thyroid gland disorders.

World Health Organization/ Centre for Disease Control [WHO/CDC] (2008) defined anaemia as a condition in which there is an inadequate number of red blood cells or an inadequate amount of haemoglobin in red blood cells. Anaemia impairs blood oxygen transport resulting in reduced physical and mental capacity. Haemoglobin is the red pigmented protein in red blood cells that carries oxygen to the brain, muscular system, immune system and other parts of the body. Iron, folic acid and other vitamins and minerals are required for the formation of haemoglobin.

Long standing pallor signifying chronic anaemia is often an unclear sign of an underlying thyroid gland disorder. Anaemia is often the first sign of hypothyroidism. Diagnosis of hypothyroidism should be considered in every case of anaemia with

uncertain etiology because sometimes signs of hypothyroidism are not always evident. In the absence of thyroid hormone, anaemia frequently develops. Complete correction of anaemia often requires restoration of thyroid function as well as specific haematinic therapy. Continued attention to haematological status is essential in the treatment of patients with thyroid disease (Antonijević, Nesovic, Trbojevic, and Milosevic, 1999).

Studies related to occurrence of anaemia and thyroid disorder have been few and not extensively studied. This was noted by Gianoukakis, Leigh and Richards (2009) who conducted a study on anaemia occurring in Graves' disease.

A study by Helfand (2004) noted that subclinical thyroid disorder can be diagnosed by thyroid function tests before symptoms and complications occur. Subclinical thyroid disorder is viewed as a risk factor for developing hyperthyroidism and hypothyroidism. Early detection to identify and treat patients with subclinical thyroid disorder before they develop hyperthyroidism and hypothyroidism can assist in the treatment of thyroid disorders.

The study aims at investigating the effect of thyroid disease on haemoparameters associated with subtle clinical manifestations in females of reproductive age, in order to promote early detection and initiate therapy of thyroid disease.

1.2 Statement of the Problem

Patients are often diagnosed for other diseases unrelated to thyroid disorder due to difficulties in the early detection of thyroid disorders. Analysis of haemoparameters associated with the unclear clinical manifestations has not been documented locally.

Lack of early detection may lead to severity of generalized symptoms such as weight loss and muscle pain resulting in abnormal levels of urea and creatinine. Oedema caused by accumulation of fluid in peripheral extremities of the body due to abnormal plasma electrolyte levels. Mannangi *et al*., (2015) noted that the effect of thyroid disorder on electrolytes is not well established and the underlying mechanism is unclear. Thyroid disease is often overlooked as a possible cause. The presence of pallor and constant tiredness a condition associated with low haemoglobin is often overlooked as an early manifestation of subclinical thyroid disorder occurring prior to the classical manifestations seen in advanced thyroid disease.

1.3 Justification of the Study

This study was set to investigate the effect of thyroid disorder on haemoparameters in reproductive aged women inorder to improve the health status and assist in reducing maternal morbidity. Classification of thyroid disease into subclinical and overt stage by Ladenson *et al*., (2000) may assist in identifying early forms of the disease before it progresses to the overt or advanced stage, which may result in increased severity of the disease.

Lazarus (2014) reported that the presence of thyroid disease in females prior to conception is likely to progress during pregnancy. Pregnancy may affect the course of thyroid disorders and thyroid diseases may affect the course of pregnancy.

A study conducted by Hill, Mwangi and Wagana (2004) in a rural hospital in Kenya noted that the morbidity rate of thyroid disorder was 3.6% they also noted that information on the burden of thyroid disease in Kenya is scanty and that few previous studies exist regarding thyroid diseases in East Africa. This study would help monitor and initiate prompt management of thyroid disease.

1.4 Objectives

1.4.1 General Objective

To evaluate the effect of thyroid gland disorder on haemoparameters in female patients at Moi Teaching and Referral Hospital.

1.4.2 Specific Objectives

1. To determine the effect of thyroid gland disorders on plasma urea and creatinine levels in female patients.
2. To determine the effect of thyroid gland disorders on plasma electrolyte levels (Na^+, K^+ and Cl^-) in female patients.
3. To determine the effect of thyroid gland disorders on haemoglobin levels in female patients.
4. To evaluate the levels of plasma thyroid stimulating hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) in female patients with thyroid gland disorders.
5. To determine the relation between thyroid stimulating hormone with plasma urea and creatinine, electrolytes and haemoglobin levels in female patients with thyroid gland disorders.
6. To compare the level of haemoparameters between female patients with thyroid gland disorders and those without thyroid gland disorders.

1.5 Research Question

1. What are the urea and creatinine plasma levels in women with thyroid gland disease?
2. What are the plasma electrolyte levels in women with thyroid gland disease?
3. What is the haemoglobin level in women with thyroid gland disease?

4. What is the variation in the levels of plasma thyroid hormone in women with thyroid disease?
5. What is the relation between thyroid stimulating hormone with plasma urea and creatinine, electrolytes and haemoglobin levels in women with thyroid gland disease?

1.6 Hypotheses

H_{o-} There is no association between thyroid gland disease with plasma urea and creatinine levels in women patients at MTRH.

H_{o-} There is no association between thyroid gland disease with plasma electrolyte levels in women patients at MTRH.

H_{o-} There is no association between thyroid gland disease with haemoglobin levels in blood from women patients at MTRH.

H_{o-} There is no association in the level of haemoparameters in female patients with thyroid gland disorders and those without thyroid gland disorders.

H_{α}- There is an association between thyroid gland disease with urea and creatinine plasma levels in women patients at MTRH.

$H_{\alpha-}$ There is an association between thyroid gland disease with plasma electrolyte levels in women patients at MTRH.

$H_{\alpha-}$ There is an association between thyroid gland disease with haemoglobin levels in blood from women patients at MTRH.

$H_{\alpha-}$ There is an association in the level of haemoparameters between female patients with thyroid gland disorders and those without thyroid gland disorders.

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy of the Thyroid Gland

The thyroid gland is a large H-shaped organ, positioned deep in the anterior sternothyroid and sternohyoid neck muscles, at the level of C5-T1 (Marieb and Hoehn, 2004). The gland consists of two bulky lateral lobes connected by a thin isthmus, located below and anterior to the larynx (Kumar *et al*., 2008). Each lobe is approximately 2.0 to 2.5 cm in thickness and its diameter is approximately 4 cm in length. The thyroid is one of the largest endocrine organs weighing approximately 15 to 20 grams. Thyroid tissue is confined to and present in all vertebrates (Larsen, Terry, Martin-Jean, and Ian, 2008).

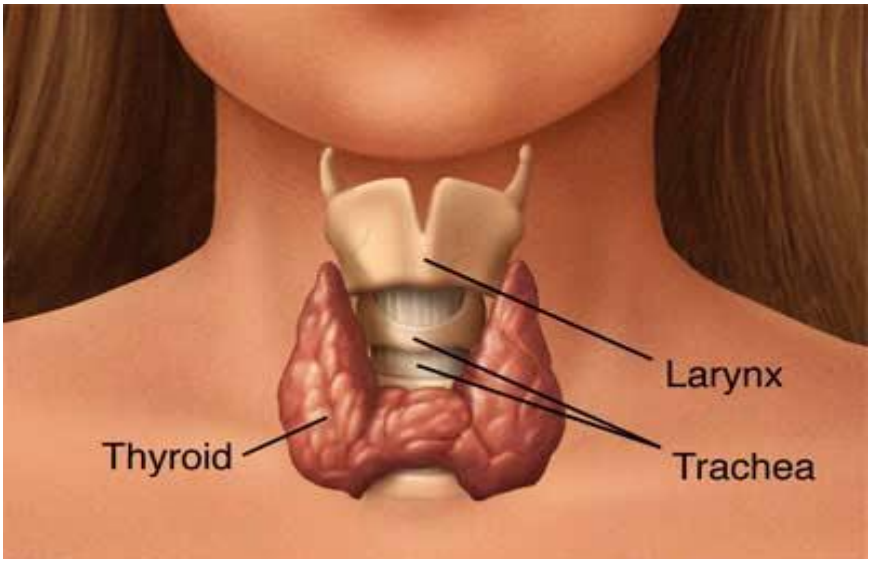

Figure 1: Structural Position of the Thyroid Gland
(Source:Thyroid Gland. Endocrine support supplement. (n.d.). Retrieved January 30, 2014, from http:/www. Tuberose.com

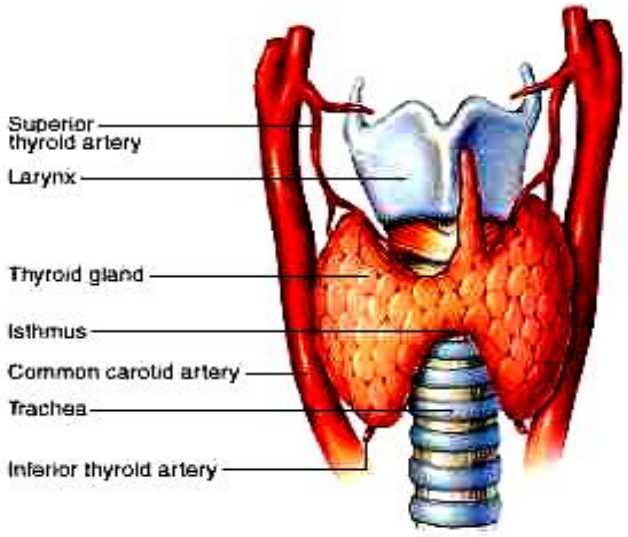

Figure 2: Anatomy of the Thyroid Gland
(Source: Thyroid gland and the Thyroid hormone: Junji Takano)

The butterfly-shaped thyroid gland is located just inferior to the larynx (voice box). It is composed of right and left lateral lobes, one on either side of the trachea, that are connected by an isthmus anterior to the trachea. About 50% of thyroid glands have a small third lobe known as the pyramidal lobe. It extends superiorly from the isthmus. The normal mass of the thyroid is about 30 g (Tortora and Derrickson, 2012).

Microscopic spherical sacs called thyroid follicles make up most of the thyroid gland. The wall of each follicle consists primarily of cells called follicular cells, most of which extend to the lumen of the follicle. A basement membrane surrounds each follicle. When the follicular cells are inactive, their shape is low cuboidal to squamous, but under the influence of TSH they become active in secretion and range from cuboidal to columnar in shape. The follicular cells produce two hormones: thyroxine or T_4, because it contains four atoms of iodine, and triiodothyronine or T_3, which contains three atoms of iodine. T_3 and T_4 together are also known as thyroid

hormone. A few cells called parafollicular cells or C cells lie between follicles. They produce the hormone calcitonin, which helps regulate calcium homeostasis. From the apex of the follicular cell, numerous microvilli extend into the colloid. It is at or near the surface of the cell that iodination, exocytosis, and the initial phase of hormone secretion occur (Tortora and Derrickson, 2012).

Plate 1: Histological cross-section of the normal thyroid gland (Source: Andrei Gunin, Atlas of Histology Images).

Mag x 450

1 - wall of the thyroid follicle, built from thyrocytes

2 - cavity of the thyroid follicle, filled with colloid

3 - blood vessel

4 - parafollicular cells (C-cells)

2.2 Thyroid Hormone

Approximately 93% of the metabolically active hormones secreted by the thyroid gland are thyroxine and 7 % triiodothyronine. Eventually almost all the thyroxine is converted to triiodothyronine in the tissues. Triiodothyronine is approximately four times more potent than thyroxine, but is present in blood at much smaller quantities and persists for a much shorter time than thyroxine. The thyroid gland also secretes the hormone calcitonin (Guyton and Hall, 2006).

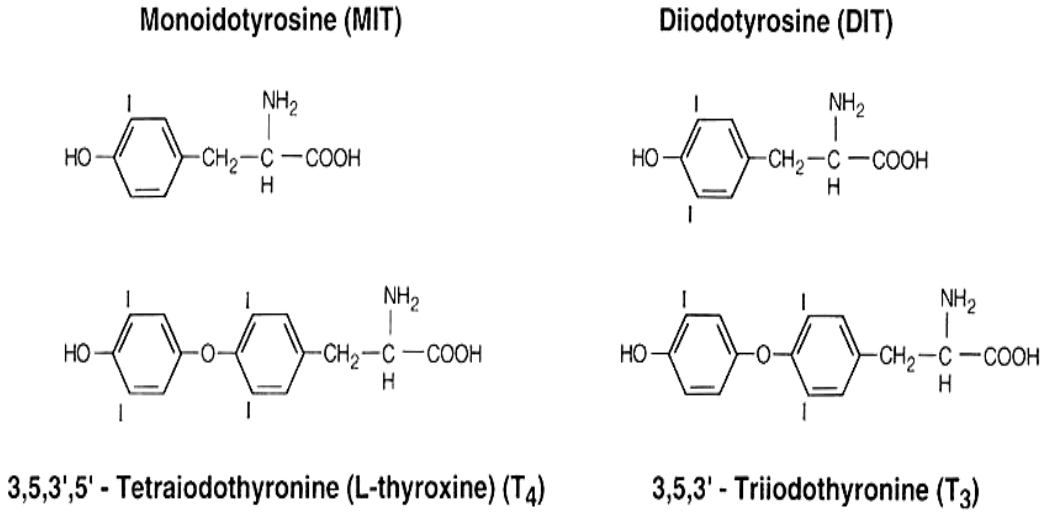

Figure 3: Figure 3: Chemical structure of the Thyroid hormone. "Thyroid Metabolic Hormone".
(Source: Textbook of Medical Physiology: Guyton).

2.3 Thyroid Hormone Synthesis

2.3.1 Requirements

Formation of normal quantities of thyroid hormone requires the availability of adequate quantities of exogenous iodine to allow thyroidal uptake of approximately 60 to 70 ug daily. At least 100 ug of iodine is required per day to eliminate all signs of iodine deficiency.

Table 1: Recommended and typical values for dietary iodine intake

(Source: Iodine and the Synthesis and Secretion of Thyroid Hormone. William's Textbook of Endocrinology: Kronenbug)

Recommended Daily Intake mcg/day(micrograms/day)	
Adult	150
During pregnancy	200
Children 1-8 years 9-13 years	9 120
Teens 14-18 years	150

Benoist, Anderson and Takkouche (2003) reported dietary intake of iodine varies throughout the world depending on the iodine content of soil and water and on dietary practice. Iodine intake also varies among individuals and in the same individuals from day to day. Iodine may also enter the body through medications, diagnostic agents, dietary supplements and food additives. Iodine is common in mountainous regions.

Iodides ingested orally are absorbed from the gastrointestinal tract into the blood and used for the synthesis of the thyroid hormone. Iodine removed from circulating blood by cells of the thyroid gland and used for synthesis of thyroid hormone (Guyton and Hall 2006).

The concentration of iodide is low. A mechanism is required for the thyroid cell to concentrate the required amounts of iodide. In a normal gland, the iodide pump concentrates the iodide to about 30 times its concentration in the blood (Larsen *et al*., 2008).

The basal membrane of the thyroid cell has the specific ability to pump the iodide actively to the interior of the cell, accomplished by a membrane protein pump called the sodium-iodide symporter (NIS) in a process known as iodide trapping. Decrease

in sodium ions may affect the electrochemical gradient and hence iodide atom uptake. A number of families identified have various mutations in the sodium-iodide symporter gene are associated with congenital hypothyroidism and as iodide transport defect (Dohan, Deta and Paroder, 2003).

2.3.2 Thyroid Hormone Synthesis

The first essential step in the formation of the thyroid hormone is conversion of the iodide ions to an oxidized form of iodine, which is then capable of combining directly with the amino acid tyrosine. Iodination of the tyrosines in thyroglobulin takes place within the colloid. Each molecule of thyroglobulin contains about 130 tyrosine amino acids, and they are the major substrates that combine with iodine to form the thyroid hormone. Thus, the thyroid hormone forms within the thyroglobulin molecule. Each thyroglobulin molecule contains up to 30 iodinated tyrosine molecules, but only 3 thyroxines and even fewer triiodothyronines. In this form, the thyroid hormone is stored in the follicles in an amount sufficient to supply the body with its normal requirements of thyroid hormone for 2 to 3 months (Guyton and Hall, 2006).

Within the thyroid, iodide participates in a series of reactions that lead to the synthesis of the active thyroid hormone. This involves oxidation of iodide and incorporation of the resulting intermediate into the hormonally inactive mono-iodotyrosine (MIT) and diiodotyrosine (DIT) a process known as organification. The formation of iodotyrosines occurs within thyroglobulin (Tg). Oxidation of thyroidal iodide is catalyzed by the enzyme thyroid peroxidase (TPO). The rate of organic iodination is dependent on the degree of thyroid stimulation by thyroid stimulating hormone (Costanzo, 2007).

The MIT and DIT formed via oxidation and organic binding of iodide are precursors of the hormonally active T_4 and $T_{3,}$ synthesis of T_4 from DIT requires the TPO -

catalyzed fusion of two DIT molecules to yield a structure with two diiodinated tyrosine residues MIT + DIT = T_3, DIT + DIT = T_4 (Larsen *et al*., 2008).

The first step in thyroid hormone release is the endocytosis of colloid from follicular lumen by two processes, macropinocytosis by pseudopods formed at the apical membrane and micropinocytosis by small coated vesicles at the apical surface. TSH stimulates both processes. Thyroxine and triiodothyronine must first be cleaved from the thyroglobulin molecule (Tg) and then these free hormones are released. T_4 is produced entirely by the thyroid gland, 20% of T_3 is produced directly by the thyroid gland by coupling of MIT and DIT. 80% of T_3 is produced by the deiodination of T_4 in peripheral extra-thyroidal tissues (mainly liver and kidney). T_4 is converted to T_3 (the biologically active metabolite) by *5′- deiodination* (Amir and Karum, 2009).

Deiodination is mediated by cellular type 1 deiodinase (DIO) which mediates hormone metabolism. In the hypothalamus and pituitary, type 2 deiodinase (DIO2) converts T_4 to T_3. Hepatic type 1 deiodinase (DIO1) mediates peripheral hormone conversion contributing significantly to circulating T_3 levels, in contrast type 3 deiodinase (DIO3) converts T_4 to inactive metabolites reverse T_3 (rT_3) and T_2 thus limiting thyroid hormone action (Gurnell, Visser and Beck-Peccoz, 2010).

The cleaving of iodine from the monoiodotyrosine and diiodotyrosine by the deiodinase enzymes makes it available again for recycling within the gland for forming additional thyroid hormone. Iodothyronine deiodinase enzymes (D1, D2, and D3) are involved in the conversion of thyroxine (T_4) to active triiodothyronine (T_3) and the inactivation of both thyroid hormone (Ronald and Csaba, 2005). In the congenital absence of this deiodinase enzyme, many persons become iodine deficient because of failure of this recycling process (Guyton and Hall, 2006).

2.4 Negative feedback control of thyroid hormone

Thyrotropin releasing hormone (TRH) from the hypothalamus and thyroid stimulating hormone (TSH) from the anterior pituitary stimulate synthesis and release of thyroid hormone. Low blood levels of T_3 and T_4 or low metabolic rate stimulates the hypothalamus to secrete TRH. Thyrotropin releasing hormone then enters the hypophyseal portal veins and flows to the anterior pituitary where it stimulates thyrotrophs to secrete TSH. Thyroid stimulating hormone stimulates all aspects of thyroid follicular cell activity including iodide trapping, hormone synthesis and secretion and growth of the follicular cells. The thyroid follicular cells release T_3 and T_4 into the blood until the metabolic rate returns to normal. An elevated level of T_3 inhibits release of TRH and TSH (negative feedback inhibition) (Gerard and Bryan, 2012).

2.5 Regulation of Thyroid Hormone Secretion

The thyroid participates with the hypothalamus and pituitary in feedback control mechanism. In addition, there is an inverse relationship between the glandular organic iodine level and the rate of hormone formation. Such mechanisms help to stabilize the rate of hormone synthesis despite fluctuations in the availability of iodine (Larsen *et al*., 2008).

To maintain normal levels of metabolic activity in the body the right amount of thyroid hormone is secreted at all times. TSH is an anterior pituitary hormone, which is the major regulator of the morphologic and functional states of the thyroid gland. TSH is a glycoprotein with a molecular weight of about 28,000. An important early effect of TSH is to initiate proteolysis of the thyroglobulin, which causes release of thyroxine and triiodothyronine into the blood within thirty minutes. TSH binds with

specific TSH receptors on the basal membrane surfaces of the thyroid cell (Guyton and Hall, 2006).

Most of its effects result from activation of the "second messenger" cyclic adenosine monophosphate (cAMP) system of the cell. Thyrotropin releasing hormone (TRH) secreted by nerve endings in the median eminence of the hypothalamus controls anterior pituitary secretion of TSH. From the median eminence, TRH is transported to the anterior pituitary by way of the hypothalamic hypophysial portal blood. TRH directly affects the anterior pituitary gland thyrotropic cells to increase their output of TSH (Guyton and Hall, 2006).

2.6 Physiological Functions of Thyroid Hormone

The main function of the thyroid gland is the production of the thyroid hormone. Thyroid hormone consists of two hormones that is triiodothyronine (T_3) and thyroxinine (T_4). The main function is to increase the metabolism of macronutrients consisting of carbohydrates, fats and proteins and to regulate metabolism in most tissues of the body (Marieb, 2004; Moore, 2006).

Thyroid hormone achieves this in two ways by increasing the oxidation of glucose thereby reducing the glucose levels in the body. This increased oxygen consumption and subsequent heat production results in increased basal metabolic rate (Drake, 2008; Moore, 2006).

Thyroid hormone causes increased blood flow and cardiac output. The heart rate increases under the influence of thyroid hormone. Thyroid hormone has a direct effect on the excitability of the heart, which in turn increases the heart rate. This effect is of particular importance because the heart rate is one of the sensitive physical signs clinicians use in determining whether a patient has excessive or diminished thyroid hormone production. Hyperthyroidism often results in diarrhea. Lack of

thyroid hormone can cause constipation. Thyroid hormone increases the rapidity of cerebration, which describes the increase in thought, or thinking process, the lack of thyroid hormone decreases this function. The hyperthyroid individual is likely to have extreme nervousness and many psychoneurotic tendencies, such as anxiety complexes, extreme worry and paranoia (Guyton and Hall, 2006).

2.7 Thyroid Disorders

Thyroid disorder is a general term representing several different diseases involving the thyroid hormone and the thyroid gland. Thyroid disorders are classified into two major categories, hyperthyroidism and hypothyroidism, depending on whether plasma thyroid hormone levels (T_4 and T_3) are increased or decreased respectively. Thyroid disease may be classified on the bases of etiologic factors or physiologic abnormalities (De Ruiter, 2002).

Manifestation of thyroid disease is usually due to excessive or insufficient production of thyroid hormone. This is exhibited by local symptoms in the neck (goiter) or in cases of Graves' disease, ophthalmopathy (disease of the eye) or dermopathy (skin disease). Physical examination of the neck is performed under adequate light with the neck relaxed in a neutral or slightly extended position to allow the neck muscles to relax. The patient is provided with a cup of water to facilitate swallowing. The position of the trachea is noted and if a swelling is present, a determination is made as to whether it moves with swallowing (Larsen *et al*., 2008).

Movement on swallowing is a characteristic of the thyroid gland because it is ensheathed in the pretracheal fascia. This feature distinguishes goiter from other neck swellings. On palpation, an increase in thickness of the isthmus or a firm texture suggests the presence of thyroid enlargement due to Hashimoto or Graves' disease.

Auscultation of the neck may confirm the increased vascularity of an enlarged hyperactive gland suggesting Graves' disease (Larsen *et al*., 2008).

2.8 Causes of Hypothyroidism

Hypothyroidism is a disorder manifested by decreased thyroid hormone synthesis and secretion. Hypothyroidism maybe classified according to the causes as being primary in which the cause originates from the thyroid gland. It maybe secondary in that the cause originates from the anterior pituitary gland or tertiary when the hypothalamus is involved resulting in decreased secretion of thyrotropin releasing hormone (Longmore, 2007). Hypothyroidism can be categorized as subclinical hypothyroidism this describes patients who have an elevated TSH and a normal thyroid hormone level, patients often display unclear signs and symptoms of the disease. Overt hypothyroidism describes patients who have an elevated thyroid stimulating hormone (TSH) and low thyroid hormone level, patients display clear signs and symptoms of the disease and hence a diagnosis can easily be made (Helfand, 2004).

Primary hypothyroidism includes autoimmune hypothyroidism, described as a type 2 hypersensitivity reaction in which the body's own immune system attacks the thyroid gland. This results in decreased synthesis and secretion of thyroid hormone. Autoimmune hypothyroidism can be diagnosed by detection of plasma thyroid auto-antibodies (Nilsson, 2001).

There are two main types of autoimmune hypothyroidism, Hashimoto's thyroiditis and atrophic thyroiditis. Thyroiditis is an inflammation and not an infection of the thyroid gland. In Hashimoto's thyroiditis auto-antibodies are directed against thyroperoxidase enzymes that catalyze iodide oxidation, transport into the follicles and binding to tyrosine residues and also against thyroglobulin. Hashimoto's thyroiditis is the most common cause of hypothyroidism. Hashimoto's thyroiditis is

more prevalent in areas with a high dietary iodine intake. Smoking increases the risk of the disease (Nilsson, 2001).

Kumar and Clark (2003) pointed out that atrophic thyroiditis is the most common presentation of severe hypothyroidism and it manifests with severe symptoms such as myxoedema. It is similar to Hashimoto's thyroiditis but is differentiated from it by the absence of a goiter.

Other types of hypothyroidism include environmental and nutritional hypothyroidism, postpartum thyroiditis, De Quervain's thyroiditis, congenital hypothyroidism, post-surgical or post irradiation hypothyroidism. Postpartum thyroiditis is a self-limiting transient condition that follows pregnancy. Some women develop hypothyroidism permanently which usually manifests with hyperthyroidism followed by a hypothyroid phase. De Quervain's thyroiditis involves the formation of granuloma in the thyroid gland causing damage to the follicle cells. The thyroid gland swells rapidly, is painful, and tender (Longmore, 2007).

Congenital hypothyroidism affects the foetus and occurs because of lack of thyroid hormone in pregnant women, this causes abnormal development of the foetal nervous and skeletal systems (Marieb and Hoehn, 2004).

Post-surgical or post irradiation hypothyroidism describes hypothyroidism occurring in patients who have had a significant portion of their thyroid gland surgically removed or partly destroyed using radioiodine. Surgical or radioiodine therapy is administered as treatment for hyperthyroidism or thyroid malignancy (Drake, 2008).

In secondary hypothyroidism, a decreased secretion of TSH from the anterior pituitary gland results in decreased stimulation of the thyroid gland leading to decreased synthesis and secretion of thyroid hormone. It usually follows physical trauma to the pituitary gland, presence of a tumour or following surgery of this area

(O'Connor, 2007).The main cause of tertiary hypothyroidism is physical trauma to the hypothalamus resulting from surgery or a tumour (Longmore, 2007).

2.8.1 Clinical Manifestations of Hypothyroidism

Hypothyroid patients present with symptoms that characterize an opposite effect compared to the normal physiological function of thyroid hormone, which increases the basal metabolic rate (O'Connor, 2007).

Symptoms of hypothyroidism are due to reduced thyroid hormone these include tiredness resulting from reduced energy production in the form of adenosine triphosphate (ATP) due to decreased utilization of macronutrients (fats, carbohydrates, and amino acids) and weight gain due to reduced metabolism of macronutrients (Longmore, 2007*).*

The presence of a goiter, which is, described as an abnormal growth of the thyroid gland resulting from overstimulation of the thyroid stimulating hormone (TSH) receptors on the thyroid follicle cells. This causes increased thyroid activity, hypertrophy and hyperplasia of the follicle cells resulting in an increase in the size of the thyroid gland. The enlargement appears as a swelling in the anterior aspect of the neck. This over stimulation can either be due to increased TSH levels or due to stimulatory antibodies which bind to the TSH receptors (Collier, 2006).

Goiter is present in some forms of primary hypothyroidism in which low thyroid hormone levels cause inhibition of the negative feedback mechanism that regulates the secretion of TSH. In primary hypothyroidism in order to compensate for a low thyroid hormone, the TSH levels are elevated overstimulating the follicle cells resulting in goiter formation. In secondary and tertiary hypothyroidism, levels of TSH levels are reduced and a goiter is absent (Collier, 2006).

Myxoedema is the accumulation of hydrophilic mucopolysaccharides in the ground substance of subcutaneous tissues (the dermis), which leads to the thickening of skin. It occurs in severe adult hypothyroidism (Kahaly, 2005).

Cretinism is characterized by symptoms that include growth failure, permanent mental retardation and lack of hair and teeth. It occurs in babies with congenital hypothyroidism that results in reduced tissue growth and development (Drake, 2008).

In hypothyroidism, a TSH level is the best screening test, a normal TSH rules out primary hypothyroidism in asymptomatic patients. An abnormal TSH level is followed by determination of the levels of T_3 and T_4 hormones (Skugor and Fleseriu, 2009).

2.9 Causes of Hyperthyroidism

Hyperthyroidism results from excess thyroid function. The main causes of this include Graves' disease, toxic multinodular goiter and adenomas. Hyperthyroidism can be categorized as subclinical hyperthyroidism, a term used to describe conditions characterized by a low TSH and normal levels of circulating thyroid hormone (thyroxine and triiodothyronine). Overt hyperthyroidism refers to low TSH and an elevated thyroxine or triiodothyronine (Helfand, 2004).

Hyperthyroidism can be classified as primary, secondary and tertiary types. Primary hyperthyroidism is defined as excessive thyroid hormone secretion due to diseases affecting the thyroid gland directly. Some of the causes include Graves' disease, which is the most common form of hyperthyroidism (Longmore, 2007).

In Graves' disease, the patient produces Ig G antibodies complementary to the TSH receptor found on the cell surface membrane of thyroid follicle cells. The antibody hyper-stimulates the receptor. This triggers follicle cell hypertrophy resulting in the

formation of a toxic goiter and causes excessive thyroid hormone synthesis or secretion (thyrotoxicosis) (Fauci *et al*., 2008).

Toxic multinodular goiter is the second most common cause of hyperthyroidism after Graves' disease and is defined as the presence of two or more thyroid nodules secreting excess thyroid hormone and causing hyperthyroidism. Hyperthyroidism can be potentiated by iodine containing drugs (Skugor and Fleseriu, 2009).

Iodide-induced thyrotoxicosis (Jod-Basedow Phenomenon) refers to induction of hyperthyroidism after a large load of iodine in form of drugs is administered to a susceptible patient. Thyroiditis of all types causes inflammation of thyroid tissue in acute and subacute thyroiditis. Thyroid tenderness and neck pain are often present. Thyroiditis reduces iodine uptake in thyroid cells (Skugor and Fleseriu, 2009).

Secondary hyperthyroidism includes TSH secreting pituitary adenoma, thyroid hormone resistance syndrome, chronic gonadotropin secreting tumours and gestational thyrotoxicosis (Fauci *et al*., 2008).

2. 9. 1 Clinical Manifestation of Hyperthyroidism

Hyperthyroidism is defined as the over-activity of the thyroid gland, which results in excessive synthesis, and secretion of thyroid hormone. More than 99% of hyperthyroidism cases are primary and are caused directly due to pathology of the thyroid gland. Secondary hyperthyroidism is a rare condition that occurs when there is excessive TSH in the circulation, which over-stimulates the thyroid gland. The main cause for secondary hyperthyroidism is a benign anterior pituitary tumour, which hyper-secretes TSH (Nussey and Whitehead, 2001).

The clinical manifestations of hyperthyroidism are directly related to increased thyroid hormone secretion. The signs and symptoms include weight loss and increased appetite due to increased metabolism, tremors due to increased thyroid

hormonal levels, which hyper-stimulate the nervous system so that neurons are much more excitable. Elevated thyroid hormone causes heat intolerance, raised body temperature and excess sweating. Raised thyroid hormone causes increased heart rate because of tachycardia following increased stimulation of the heart's β-adrenergic receptors (Nussey and Whitehead, 2001).

The presence of a goiter is a common finding in both primary and secondary hyperthyroidism. Goiter formation results from antibodies that stimulate TSH receptors as in Graves' disease and formation of a tumour within the thyroid gland or inflammation of the thyroid (Kumar and Clark, 2003).

2. 10 Effect of Thyroid Hormone on Renal Physiology

Thyroid hormone plays a crucial role in the development and physiology of the kidney. The kidney is also involved in the metabolism and elimination of thyroid hormone. Thyroid hormone influence water and electrolyte balance in different compartments of the body (Gattineni, Dagan, Dwarakanath and Baum, 2008).

The kidney plays a role in the elimination of thyroid hormone and is an important target organ for thyroid hormone action. Thyroid disorder causes changes in glomerular and tubular functions and electrolyte and water homeostasis. Thyroid hormone influence renal functions by prerenal and renal effects. Prerenal effects are mediated by the influence of thyroid hormone on the cardiovascular system and the renal blood flow (RBF). The renal effects are mediated by thyroid hormone, which increases the glomerular filtration rate (GFR), tubular secretory and reabsorptive processes, as well the influence on the renal tubular physiology (Basu, and Mohapatra, 2012).

Ionized form (Na^+) is the most abundant cation (positively charged particle) in extracellular fluid; essential for maintaining water balance needed to generate action

potentials. Ionized form of potassium (K^+) is the most abundant cation in intracellular fluid and is utilized to generate action potentials. Ionized form of chlorine (Cl^-) is the most plentiful anion (negatively charged particle) in extracellular fluid and is essential for maintaining water balance (Tortora and Derrickson, 2012).

Thyroid hormone influences sodium ion (Na^+) reabsorption at the proximal convoluted tubule by increasing the activity of the Na^+/K^+-ATPase (sodium ion/ potassium ion-adenosine triphosphatase) a membrane bound enzyme (Costanzo, 2007).

2.11 Effect of Thyroid Disease on Plasma Electrolytes, Urea and Creatinine

The thyroid hormone directly affects renal physiology and is mediated by cardiovascular and systemic haemodynamics that influence kidney function. Consequently, both hypothyroidism and hyperthyroidism are associated with alterations in kidney function and have relevance to its assessment. Disorders of thyroid function have been linked to development of immune-mediated glomerular injury. Alteration in thyroid hormone and thyroid hormone testing occur in patients with kidney disease (Mariani and Jeffery, 2012).

In a study carried out by Hollander, Wulkan, Mantel and Berghout (2005) he noted that hypothyroidism is accompanied by a decrease in glomerular filtration, hyponatreamia (low sodium levels in the blood) and an alteration in the ability to excrete water. Excessive levels of thyroid hormone generate an increase of glomerular filtration rate and renal plasma flow (Hollander *et al.*, 2005).

Thyroxine (T_4) is produced by the thyroid gland, triiodothyronine (T_3) the more biologically active form of thyroid hormone, is produced primarily through local deiodination of T_4 by the enzyme 5′-deiodinase in other tissues including the kidney. The kidney contains the D1 isoform of this enzyme that becomes less active in

uraemia, which is described as elevated levels of urea in the blood. This condition results from accumulation of byproducts of degradation of proteins resulting from elevated levels of thyroid hormone as seen in hyperthyroidism. Catabolic degradation of proteins in muscle results in muscle wasting clinically evident as weight loss (Mariani and Jeffery, 2012).

In a study done by Tayal, Chawla, Arora, Gupta, and Mallik, (2009) they noted that hypothyroidism can cause significant reversible changes in renal function. This include reduced sodium reabsorption in the proximal tubules, impairment in the concentrating and diluting capacities of the distal tubules, leading to hyponatreamia and a decrease in urinary urate excretion (the form in urine in which urea is eliminated from the body), due to a decrease in renal blood flow, leading to accumulation of urea in the circulation.

Murgod and Gladys (2012) pointed out that sodium and potassium are important components of the enzyme Na^+/K^+-ATPase a membrane bound enzyme that catalyzes the transport of Na^+ and K^+ across the membrane. Thyroid hormone regulates the activity of sodium-potassium pumps in tissues. Hypothyroidism causes low plasma K^+ levels. Deficiency of thyroid hormone affects the efficacy of the enzyme Na^+/K^+-ATPase resulting in accumulation of water inside the cells causing oedema. This is probably one of the mechanisms contributing to weight gain seen in hypothyroid patients. However, in another study Basu and Mohapatra (2012) reported normal plasma concentration of sodium and potassium ions.

2.12 Effects of Thyroid Hormone on Red Blood Cell Formation

The process of blood formation is known as haematopoiesis. Red blood cells are synthesized in the bone marrow. The composition of red blood cells is about 45% of total blood volume in an adult. Red blood cells compose the main cellular component

of blood. Erythropoietin a glycoprotein hormone is the major physiological stimulator of red blood cell formation in mammals. Erythropoietin production is induced by hypoxia, a state in which oxygen supply does not cover the demand of the body's tissue cells resulting in lack of oxygen. The main erythropoietin production sites are the kidney in adults and the liver in fetuses (Stuart, 2012). A mature red blood cell contains the oxygen carrying polypeptide pigment known as haemoglobin (Cheesbrough, 2007).

Thyroid hormone has major effects on oxygen consumption and metabolic rate at the cellular level. Adaptation to this increased metabolic demand is achieved by potent effects of thyroid hormone on erythropoiesis and thus blood oxygen capacity. Thyroid hormone directly increases the proliferation of erythroid progenitors (Dorgalaleh *et al*., 2013)

Thyroid hormone produces an effect by binding with their specific receptors in the membrane and nuclei of haemopoietic stem cells in the bone marrow. After T_3 and T_4 hormones bind with a receptor, erythroid stem cells go through mitosis and accelerate erythropoiesis with protein synthesis occurring in precursor cells, for the synthesis of enzymes, which catalyze haemoglobin formation (Kawa, Grymula, Paczkowska and Koziole, 2010).

In normal physiological states, thyroid hormone enhances erythropoiesis during depletion of tissue oxygen (hypoxia). This occurs by hyperproliferation of immature erythroid progenitors (stem cells) and increased secretion of erythropoietin (EPO) from the kidneys, by inducing erythropoietin gene expression by binding to the intracellular thyroid hormone receptor (TR), a member of the nuclear hormone receptor family, which acts as a transcription factor and regulates gene expression regulating erythropoiesis. Thyroid hormone also augments repletion of hypoxia

inducible factor1 (HIF-1) and then stimulate the growth of erythroid colonies (BFU-E, CFU-E) in the bone marrow. This hormone also intensify erythrocyte 2, 3 BPG **(**2, 3-bisphosphoglycerate) an inorganic phosphate produced in red blood cells. 2, 3 BPG binds to haemoglobin, lowering its affinity for O_2 facilitating the release of O_2 to body tissues (Dorgalaleh *et al*., 2013).

2.13 Effect of Thyroid Disease on Red Blood Cell Formation

Thyroid hormone is involved in haemoglobin synthesis in adults and maturation of haemoglobin in foetus and by affecting haematopoietic process. Thyroid disorders are often accompanied with decreased levels of red blood cell in the circulation. Hypothyroidism results in reduced erythropoietin production and secretion from the kidney and hypoplasia of the bone marrow a condition that describes a reduction in haematopoietic cells are suggested causes of anaemia (Squizzato, Romualdi, Buller and Gerdes, 2007).

Depressed red blood cell levels in the circulation are frequently associated with thyroid disorder, but is rarely investigated and linked to the thyroid gland (Lippi, Montagnana, Salvagno and Guidi, 2008). Thyroid hormone has a significant influence on erythropoiesis resulting in anaemia, which has been associated with decline in thyroid function (Omar *et al*., 2010). Jameson and Weetman (2010) noted that the anaemia in hypothyroidism is usually macrocytic hypochromic anaemia (large sized cell and low pigmentation concentration) of moderate severity. They also observed that anaemia in hypothyroid patients is unresponsive to therapy with iron, vitamin B12, or folic acid. The degree of anaemia is classified as being mild to moderate, with haemoglobin levels rarely less than 8 to 9 g/dl (normal reference range for Hb level 12-18g/dl [WHO/CDC 2008]).

Ford (1990) reported that anaemia was not commonly observed in patients with hyperthyroidism. He suggested that hyperthyroid patients exhibit an increase in erythropoiesis reflected in the bone marrow, which undergoes erythroid hyperplasia resulting in an increase in the total red cell mass. However, indices such as haemoglobin and red blood cell count are not elevated despite an increase in the total red cell mass due to the increase in plasma volume, which results in haemodilution. He noted that most patients exhibited minimal changes in the haemoglobin level.

A mild anaemia with low haemoglobin levels sometimes develops in patients with hyperthyroidism, the major symptom being fatigue. The mechanisms underlying these actions remain unclear (Gianoukakis, Leigh and Richards, 2009).

In a study carried out by Bashir *et al*., (2012) they noted the importance of thyroid hormone in the physiological role in human metabolism in the haematopoietic system and suggested that erythrocyte abnormalities are probably associated with thyroid disorder. They concluded that subclinical thyroid disorder is probably associated with anaemia and that patients are at risk of progressing to overt thyroid disorders and recommended that such conditions should be identified and corrected.

2.14 Thyroid Disease in Women

Many thyroid conditions are more common in women than in men. This condition is not only due to estrogen since it can occur before and after puberty. It has been suggested that there may be a genetic link to these disorders. Researchers at Mount Sinai suggested that gender predisposition is genetically mediated. Women may inherit a predisposition to thyroid disorders based on their gender (Wider, 2005).

Peat (2011) reported that excess secretion of estrogen inhibits the secretion of thyroid hormone by the thyroid gland by probably inhibiting the proteolytic enzyme, which dissolves the colloid contained in the cavity of the thyroid follicle. Progesterone has

the opposite effect of promoting the release of the thyroid hormone from the gland. At puberty, pregnancy and menopause the thyroid gland enlarges probably because of estrogen dominance. When there is an excess of any hormone in relation to the body's endocrine system, an overall imbalance develops and health problems can arise. This occurs when the body has too much estrogen and not enough progesterone to counteract it. This situation is known as estrogen dominance.

Lucille (2014) reported that the cause of estrogen dominance include excess exposure to environmental estrogens known as xenoestrogens which are found in industrial compounds, consumer products such as detergents and lotions, plastics, fertilizers, pesticides as well as food preservatives. Plant estrogens known as phytoestrogens found in soy, cabbage, synthetic estrogens such as birth control pills and negative lifestyles such as smoking, alcohol use and obesity can cause estrogen dominance.

Lazarus (2014) pointed out that in regions where the iodine supply is borderline or low, significant changes occur during pregnancy. In early pregnancy, there is an increase in renal blood flow and glomerular filtration, which lead to an increase in iodide clearance from plasma. This results in a fall in plasma iodine concentrations and an increase in iodide requirements from the diet. In addition, there is a further increment in iodine requirements, due to transplacental iodide transport necessary for iodothyronine synthesis by the fetal thyroid gland, which becomes progressively functional after the first trimester. When pregnancy takes place in women with borderline or low plasma iodine levels, it results in prolonged enhanced thyroidal stimulation that leads to goiter formation in both mother and fetus.

2.15 Evaluation of Thyroid Function

Thyroid function test is used to evaluate the thyroid's functioning and to diagnose and help determine the cause of disease. The principal hormone for diagnosing hyperthyroidism and hypothyroidism is the thyroid stimulating hormone test (National Endocrine and Metabolic Diseases Information Service [NEMDI], 2010).

Abnormal TSH is followed by determination of thyroid hormone levels. Hyperthyroidism is diagnosed by suppressed TSH and high or normal FT_4 or FT_3 (unbound thyroxine and triiodothyronine to the protein thyroid binding globulin, [TBG]) or both. The level of TSH is increased in hypothyroidism due to loss of feedback inhibition of TRH and TSH production by the hypothalamus and pituitary respectively (Skugor and Fleseriu, 2009).

Helfand (2004) indicated that measurement of plasma TSH concentration provides the most useful single screening test for hyperthyroidism because the level is reduced even at the earliest stages of the disease.

Skugor and Fleseriu (2009) also observed that TSH level is the most appropriate screening test for detecting hypothyroidism. A comprehensive history and physical examination including weight recording and blood pressure, pulse rate cardiac rhythm and a neuromuscular examination. Thyroid palpation and auscultation to determine thyroid size, nodularity and vascularity. An eye examination to detect evidence of exophthalmos and a dermatologic examination is recommended.

2.15.1 Laboratory Evaluation

Only the unbound thyroid hormone is available to tissues. T_3 is less strongly bound to the bonding protein thyroid binding globulin and therefore has a more rapid action. The binding protein thyroid binding globulin (TBG) has both storage and buffer functions. It helps to maintain the plasma free T_4 and T_3 levels within narrow limits

and ensure continuous and rapid availability of the hormone to tissues (Amir and Karum, 2009).

Total thyroid hormone levels are elevated when thyroid binding globulin are elevated due to the influence of estrogen hormone in pregnancy, oral contraceptives, hormone therapy, drugs such as tamoxifen and decreased when TBG binding is reduced as a result of androgens and nephrotic syndrome (Fauci *et al*., 2008).

Marc *et al*., (2003) also noted physiologic situations associated with a change in the plasma concentration of thyroid binding proteins such as pregnancy, non-thyroidal illness or ingestion of drugs that affect the level and/or affinity of these binding proteins. Under these circumstances, the plasma concentrations of total thyroxine (T_4) and total triiodothyronine (T_3) change parallel to the changes that occur in the thyroid hormone binding proteins. The plasma concentration of free T_4 and free T_3 are raised in hyperthyroidism and are decreased in hypothyroidism. The plasma concentrations of free thyroxine (FT_4) and free triiodothyronine (FT_3) remain normal in individuals and are described as euthyroid.

Genetic disorders and acute illness can cause abnormalities in thyroid hormone binding proteins and can interfere with thyroid hormone binding hence free thyroid hormone concentrations are easier to interpret than total thyroid hormone levels (Fauci *et al*., 2008).

Thyroid function tests aim at assessing the functional and anatomical status of thyroid gland, the test also determines the course of the disorder and monitors the patients under treatment for effectiveness and progress. The plasma TSH is the most sensitive test for detecting thyroid hormone excess or decrease. Other laboratory and isotope tests may include T_4 and T_3 radioimmunoassay (RIA), thyroid autoantibodies (TRAb)

or radioactive iodine. Scanning the thyroid evaluates suspicious structural thyroid abnormalities (AACE, 2002).

2.16 Treatment of Thyroid Gland Disorder

The first line treatment for hypothyroid disease is to treat the cause for example treating environment iodine deficiency hypothyroidism with increased supplementation of iodine. Most causes of hypothyroidism are genetic or autoimmune are incurable and as a result the mainstay treatment most patients involves thyroid hormone replacement for life. Oral thyroxine replacement is the main treatment in non-severe hypothyroidism (Kumar and Clark, 2003). In severe hypothyroidism, triiodothyronine replacement is prescribed, because T_3 is more biologically active in comparison to T_4 (Nilsson, 2001).

The aim of treatment regime is to restore the plasma thyroid hormone and subsequently the TSH levels to the normal range. This is monitored every 6 weeks through thyroid function tests and the thyroid hormone dose prescribed is adjusted according to the results. There are three treatment options for hyperthyroid patients these include anti-thyroid drugs such as carbimazole and propylthiouracil. Both of these drugs inhibit the thyroperoxidase enzyme, reducing synthesis of thyroid hormone. The purpose of anti-thyroid medication is to reduce plasma thyroid hormone levels to the normal range. Radioiodine comprises radioactive iodine 131, which is administered to the patient. This accumulates in the thyroid gland causing destruction of the gland. Subtotal thyroidectomy, this surgery is an alternative to radioiodine therapy (Dayan, 2001; Kumar, 2003).

2.17 Screening for Thyroid disease

Rugge and Balshem (2011) defined screening as the application of a test to detect a potential disease or condition in a person who has no known signs and symptoms of that condition at the time the test is done. They demonstrated that early treatment can prevent symptoms in patients who have no history of thyroid disease and no clinical findings attributed to thyroid disease, are unlikely to progress rapidly to the clinical disease. They observed that the majority of patients identified by screening had no clinical findings or symptoms.

Marc *et al*., (2003) suggested that when many of the classical signs and symptoms occur together the clinician might have a strong suspicion that the patient has thyroid disease. Patients who complain of one or two of the symptoms are likely to have abnormal thyroid function tests suggestive of thyroid disorder as those who have no complaints. This has often resulted in delay in early treatment resulting in increased severity and associated complications. Some of these complications include anaemia, oedema, heavy menstruation, miscarriages and mental retardation of the foetus.

Mariani (2012) pointed out that elevation of plasma creatinine a renal manifestation of thyroid disorder, is reversible with treatment and suggested that screening for thyroid disease should be considered in patients with unexplained elevations in plasma creatinine.

Canaris, Thomas, and Robert (2011) noted some controversy of screening in other research. These studies noted that thyroid disorder such as hypothyroidism is underdiagnosed, hence many organizations do not recommend screening citing low disease prevalence in undetected populations. Canaris, Thomas, and Robert (2011) however noted in a study that a high proportion of previously undiagnosed thyroid disease was identified in a screening health fair exercise despite the lack of

symptoms. They observed that health fairs might offer screening and information on multiple topics or focus on a certain disease.

Arriving at the correct diagnosis of thyroid disease can be challenging it requires a combination of clinical skills and reliable test results supplied by the medical laboratory. The diagnosis of overt hyperthyroidism or hypothyroidism in patients presenting with classical symptoms and diagnostic laboratory results is less of a challenge compared to those patients not presenting with symptoms described in medical textbooks, are more difficult to diagnose (Laboratory Corporation of America holdings [LabCorp], 2014).

In Kenya screening programmes and promotion of early treatment have been effective for various medical diseases such as Human Immunological Deficiency Virus (HIV), tuberculosis, malaria, diabetes mellitus, breast and cervical cancer. Institutions such as the Academic Model for Providing Access to Health Care (AMPATH) located in the premises of Moi Teaching and Referral Hospital, Uasin Gishu County, Kenya has been at the forefront in promoting primary health care through these initiatives. Screening and health promotion for thyroid disease has not been adequately addressed. This study aims at investigating the effect of thyroid disorder on haemoparameters not readily associated with its diagnosis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at the Moi Teaching and Referral Hospital (MTRH) located in Eldoret town along Nandi road in the county of Uasin Gishu in Kenya, Africa. The geographical coordinates are $0^{o}30'44$ North, $35^{o}16'50''$ East. MTRH is the second largest referral hospital in Kenya after Kenyatta National Hospital. It opened in 1917 as a cottage hospital and was later elevated to a district hospital. MTRH is currently a teaching and referral institution after the establishment of the Moi University in 1984 and subsequent establishment of the faculty of Health Sciences at the University. Eldoret has a population of about 200,000 people. The hospital has a 1000 bed capacity, the MTRH serves a catchment population of 20 million from Northern and Western Kenya and across the borders to parts of Uganda and the Southern Sudan.

3.2 Study Design

This was a cross-sectional study that involved that collection of blood samples for analysis from female patients of reproductive age diagnosed with thyroid disorder through hormonal assay, and from females of corresponding age group without thyroid disorder who constituted the euthyroid or control group. The variables consisted of plasma urea and creatinine levels, sodium ion (Na^{+}), potassium ion (K^{+}) and chloride ion (Cl^{-}) (electrolyte), haemoglobin and plasma TSH, FT_3, FT_4 levels. The study was conducted at the Moi Teaching and Referral Hospital laboratories.

3.3 Study Population

The study population consisted of female patients of childbearing (reproductive) age between 15-49 years with thyroid disorder confirmed following thyroid hormonal assay in the MTRH laboratory, after meeting the inclusion criteria. Participants were

sampled from females attending the casualty department at the hospital. The euthyroid (control) group was picked during a blood donation exercise carried out by the regional blood donation centre located in Eldoret town in the county of Uasin Gishu in Kenya. The euthyroid group tested negative for hyperthyroid and hypothyroid disease, confirmed by normal thyroid hormone levels with TSH levels at 0.3-4 mIU/L. Females confirmed with thyroid disease were further grouped into two categories based on the levels of thyroid hormone. Participants were categorized as being hypothyroid with elevated TSH and low FT_4 and FT_3 hormonal levels and hyperthyroid based on low TSH and elevated FT_4 and FT_3 hormonal levels. The patients were further classified as having subclinical or overt hypothyroidism with TSH >4mIU/L and TSH>10 mIU/L respectively (Pluta, Burke, Richard, Glass, 2010) and subclinical or overt hyperthyroidism with TSH between 0.03-0.3 mIU/L and TSH≤ 0.03mIU/L respectively (Biondi, Palmieri, and Klain, 2005).

3.4 Sampling Technique

Simple random sampling procedure was used to pick a sample population of females confirmed with thyroid gland disorder through hormonal assay. The euthyroid group consisted of females of corresponding age group not exhibiting thyroid disorder. This was done by noting down all the names of the female meeting the inclusion criteria and then randomly selecting numbers corresponding to them.

3.5 Sample Size

The sample size was determined using the Fischer formula (1991):

$$n = \frac{Z^2PQ}{d^2}$$

Where:

n= desired sample size (when population is greater than 10000, in this case it was greater than 10000)

Z= the standard normal deviate, set at 1.96, which corresponds to 95% confidence level.

P= the proportion of the target population estimated to have a particular characteristic, in this case 13 %, which is the percentage of women with thyroid gland disorder (Hill, 2004).

Q= 1.0-P

d=degree of accuracy desired, set at 0.05

$$n = \frac{(1.96)^2(0.13)\,(1-0.13)}{(0.05)^2}$$

n = 174

A similar number of reproductive aged females without thyroid gland disorder were recruited into the study.

3.6 Inclusion / Exclusion

3.6.1 Inclusion Criteria

The study included consenting females of reproductive age between 15-49 years whose thyroid status was confirmed through hormonal assays and are not on antithyroid drugs.

3.6.2 Exclusion Criteria

The study excluded female patients who declined to participate in the study, females outside the stipulated age bracket, pregnant females, those on hormonal contraceptives, positive HIV status, females with medical conditions such as

hypertension and diabetes. Females on antithyroid drugs. These conditions may affect red blood cell indices. Thyroid hormone may be elevated when thyroid binding globulin is increased due to elevated levels of estrogen in pregnancy, oral contraceptives and hormonal therapy, this may lead to transient pregnancy induced hyperthyroidism or postpartum hypothyroidism. Postpartum describes the six weeks period following delivery (Fauci *et al*., 2008). Patients on drugs for chronic as well as acute illness unrelated to thyroid disease may present with altered thyroid hormone levels (Amir and Karum, 2009) and hence may not be suitable participants for the study.

3.7 Ethical Consideration

Consent of study was obtained from the Institutional Research and Ethics Committee of Moi Teaching and Referral Hospital (IREC). Informed consent was obtained from willing participants. The identity of the respondents was kept confidential by not including names on the data collection form. No unapproved or unacceptable procedure was applied in this study. Electronic data was protected by use of password.

3.8 Data Collection

3.8.1 Collection of Specimen

Blood was collected as per Cheesbrough (2007), using sterile disposable 21 gauge needles mounted on 5 millilitres syringes and a soft tubing tourniquet was applied for not more than 2 minutes to the upper arm of the patient to enable the veins to be visible. Protective gloves were worn during the procedure. A suitable vein was selected that was sufficiently large and straight. The puncture site was cleaned using 70% ethanol, which was the optimal bactericidal concentration and allowed to dry.

The venipuncture (a vein is punctured with a needle to withdraw blood or inject a fluid) was made at the puncture site and the vacutainer was allowed to fill. The tourniquet was released after sufficient blood was collected and pressure applied to the puncture site using dry cotton wool.

5.0 millilitres of the collected venous blood was gently mixed by inverting the vacutainer 6 times to ensure thorough coating with the anticoagulant immediately after the draw. The vacutainers are coated with ethylene diamine tetra acetic acid (EDTA) an anticoagulant that prevents blood from clotting. Samples were subjected to analysis of haemoparameters that included the following indices, urea and creatinine, haemoglobin, electrolytes levels and TSH, FT_4, FT_3 hormonal assays. Whole blood was collected to determine haemoglobin levels, from which 3.0 millitres of plasma extracted by centrifugation at a rotating speed of 3000 revolutions per minute (rpm) for 5 minutes, using the centrifuge Rotofix 32 A (Hettich Zentrifugen, Germany), to determine the protein metabolites, electrolyte levels and hormonal levels. After processing and storage of blood samples the vacutainers were discarded in yellow and red coded hazardous containers for disposal of infectious material and needles disposed by incineration.

3.8.2 Measurement of haemoglobin levels

The level of haemoglobin was measured to detect anaemia and its severity. A complete blood count was performed using the MINDRAY AUTO HEMATOLOGY® ANALYZER (MODEL No. BC-5500 China), using venous whole blood extracted and stored in vacutainers containing EDTA. The machine functions in the automated mode. 2.0 millitres of whole blood in the EDTA vacutainers was mixed using an electrical mixer to allow adequate coating of blood with the

anticoagulant and placed in racks on a loader on. The samples were automatically fed into the machine and the generated results printed.

3.8.3 Measurement of plasma urea and creatinine levels

Collected venous whole blood into EDTA vacutainers was subjected to centrifugation to extract plasma at a rotating speed of 3000 revolutions per minute (rpm) for 5 minutes, using the centrifuge Rotofix 32 A (Hettich Zentrifugen, Germany). The COBAS INTEGRA 400® plus analyzer (Roche Diagnostics, U.S.A) was used to test for urea and creatinine levels. Samples of 1.0 millitre were automatically transferred from a sample tube. The test principle of Ion Selective Electrode was used to obtain measurements. Results were generated automatically.

3.8.4 Measurement of plasma electrolyte levels (sodium-Na^+, chloride-Cl^- and potassium-K^+)

Electrolyte levels comprising sodium ions potassium ions and chloride ions were analyzed using the EASYLYTE® plus Analyzer (Medica, Massachusetts, USA). 1.0 milliliter of plasma was placed on an Easy Sampler containing 24-position sample tray that included 21 sample positions, one stat position and two quality control positions. The sample tray holds 0.5-2.0 millitres sample cups. The EasyLyte printed the results for each sample loaded in the sample tray.

3.8.5 Measurement of plasma TSH, T_3 and T_4 hormonal levels

Thyroid stimulating hormone (TSH) testing is the first test to perform and remains the single best test of thyroid function. Thyroid stimulating hormone testing is the preferred approach because TSH is central to the negative-feedback system and small changes in plasma thyroid hormone causes amplification in TSH secretion. Plasma TSH test should be carried out in all patients who have non-specific manifestations, are asymptomatic and in whom the diagnosis is not clear. In patients whose TSH

levels are abnormal, T_3 and T_4 levels should be determined. Free T_3 and T_4 level estimation is preferred over total T_3 and T_4 estimation because these hormones are extensively (>99%) bound to plasma proteins and only the unbound forms are active (Shashank, 2011).

As per Pishtaz Teb Diagnostics method. The plasma thyroid stimulating hormone (TSH), triiodothyronine (T_3) and thyroxine (T_4) was measured using TSH, T_3 and T_4, Elisa-Kit (Pishtaz TEB Diagnostic kit, Cat No. PT-TSH-96) as per Pishtaz Teb Diagnostics method purchased from European authorized representative: ID Consulting Services Ltd. Korbach/Germany.

A modified assay procedure was as follows. The microtiter wells were coated with Anti TSH, FT_3 or FT_4. The samples were added into the microtiter wells using a precision pipette and tested for TSH, FT_3 and FT_4 antigen, which form an antigen–antibody complex (Ag-Ab complex) on contact. A conjugate of TSH, T_3 or T_4 linked to an enzyme (Horseradish peroxidase) was then added to the samples which was later incubated in an ABBOTT Commander Dynamic Incubator for 1 hour at an average room temperature of 23^{o}C, resulting in the formation of Ag-Ab-conjugate complex. During incubation the free TSH, FT_3, and FT_4 and their conjugated forms compete for the limited binding sites on the wells. The first six wells constituted the standards.

The next procedure involved washing using the BioTek® EL x50 Automatic microplate strip washer 12 well (U.S.A). The wells are washed with reconstituted buffer solution in which 20 millitres of buffer solution is added to 100 millitres of distilled water at a pH of 7.2. The microtiter wells held by a 96 well strip holder (that is 90 Ag-Ab conjugate and 6 standards) were placed on the loading rack of the automated washer which was programmed to run 5 washes for 10 seconds at the end

of which the wells appeared clear. The wells were shaken unto absorbent gauze to remove residual wash droplets. The purpose of the wash out was to remove any excess substances not used in the reaction such as unbound TSH, FT_3 and FT_4, other hormones, the washout served to maintain the pH at 7.2.

Addition of a 100 ul solution of chromatogen-substrate was added to the wells and incubated for 15 minutes in the dark. The substrate is a colouring agent, which reacts with the Ag-Ab conjugate complex to give a blue colour. The reaction/colour development was stopped using the stop solution consisting of 100ul of 1molar sulphuric acid (H_2SO_4). The colour changed from blue to yellow. The colour intensity was inversely proportional to the intensity of the amount of TSH, FT_3 and FT_4 present in the sample. The wells containing the samples were then loaded onto the loading tray of the spectrophotometer and underwent analysis. The absorbance was measured using the BioTek® spectrophotometer ELISA reader (U.S.A) at a wavelength of 450 nm, which automatically generated results.

3.9 Data Analysis

The data obtained was analyzed using SPSS version 20 (2011). Due to its skewed distribution data was summarized and presented using median and inter-quartile range, the Kruskal Wallis non-parametric test was used to compare differences in plasma electrolyte level plasma urea and creatinine level, haemoglobin and hormone levels between females with thyroid disorder and the euthyroid group. Due to skewness in the measures, Spearman correlation test was used to compare female participants with thyroid gland disorder and those without thyroid gland disorder. A p value of <0.05 was considered significant. A p value of <0.01 was considered highly significant. Results were presented in form of tables.

CHAPTER FOUR

RESULTS

Table 2: Plasma urea and creatinine level in hyperthyroid, hypothyroid and euthyroid (control) groups

Group	Creatinine(μmol/L) Median (IQR)	Urea (mmol/L) Median (IQR)
Hyperthyroid	76(67.5, 80)	6.5(4.3, 8.7)
Hypothyroid	68(57, 80)	6.2(4.4, 7.1)
Euthyroid	62(52, 69)	3.8(3.1, 5.3)
P-value	**<0.001**	**<0.001**

4.1 Inter-quartile range for plasma urea and creatinine levels in the hyperthyroid, hypothyroid and euthyroid groups

Females with hyperthyroid and hypothyroid gland disorder had higher plasma levels of creatinine and urea compared to the euthyroid participants ($p<0.001$). The results showed a median (IQR) of creatinine and urea at 76 (67.5, 80) and 6.5 (4.3, 8.7) in hyperthyroid patients and median (IQR) of 68 (57, 80) and 6.2 (4.4, 7.1) in hypothyroid patients. The euthyroid group had a median (IQR) of 62 (52, 69) and 3.8 (3.1, 5.3).

Table 3: Plasma electrolyte levels for sodium ions, potassium ions and chloride ions (Na^+, K^+ and Cl^-) in the hyperthyroid, hypothyroid and euthyroid (control) groups

Group	Na^+ mmol/L Median (IQR)	K^+ mmol/L Median (IQR)	Cl^- mmol/L
Hyperthyroid	136.8(135.3, 140.3)	4.2(3.6, 4.4)	102.9(99.2, 105.3)
Hypothyroid	137.5(135.4, 141.0)	3.9(3.6, 4.6)	103(99.3, 104.7)
Euthyroid	139.2(137.6, 142.8)	4.0(3.7, 4.4)	102(99.4, 104.2)
p-value	**<0.001**	**0.472**	**0.303**

4.2 Inter-quartile range for plasma electrolyte levels in the groups

There was no difference in the levels of Na^+, K^+ and Cl^- between the two groups. The levels remained within the normal range.

Table 4: Haemoglobin level in hyperthyroid, hypothyroid and euthyroid (control) groups

Group	Haemoglobin g/L Median (IQR)	P-value
Hyperthyroid	11.5(10.7, 12.5)	**<0.001**
Hypothyroid	10.2(11.5, 13.0)	
Euthyroid	12.7(11.9, 13.8)	

4.3 Inter-quartile range for haemoglobin levels in the groups

Females with thyroid disorder exhibited low haemoglobin levels. Females with hypothyroidism had lower haemoglobin levels among the groups with a median (IQR) of 10.2 (11.5, 13.0) ($p<0.001$). Hyperthyroid females had a median (IQR) of 11.5 (10.7, 12.5) ($p<0.001$).

4.4 Frequency levels in females with subclinical and overt hypothyroidism and hyperthyroidism

Hyperthyroid female patients presenting with subclinical hyperthyroidism were 29 (39.72%) while 45 (60.8%) presented with overt hyperthyroidism.

Hypothyroid female patients who presented with subclinical hypothyroidism were 53 (53.0%) while 47 (47.0%) had overt hypothyroidism.

4.5 Correlation

The plasma TSH levels in the hyperthyroid, hypothyroid and euthyroid groups was correlated with plasma urea and creatinine, electrolytes and haemoglobin levels in the groups. Spearman's rho correlation test was used to calculate the correlation coefficient.

Table 5: Hyperthyroid: Correlation between plasma TSH and parameters

	Group		TSH	Urea	Creatinine	Na^+	K^+	Cl^-	Hb
TSH	Hyper	Correlation Coefficient	1.000	-.518*	-.564*	.110	.145	.131	.227
		N	74	74	74	74	74	74	74

* Correlation is significant at the 0.05 level (2-tailed).

4.5.1 Correlation between plasma TSH, plasma urea and creatinine, electrolytes and haemoglobin levels in hyperthyroid female participants

A significant negative correlation was noted between TSH and urea and creatinine (r= -0.518, -0.564). No significant correlation was noted between TSH and Na^+, K^+ and Cl^- ions. An insignificant correlation was observed between TSH and Hb (r= 0.227).

Table 6: Hypothyroid: Correlation between plasma TSH and parameters

	Group		TSH	Urea	Creatinine	Na^+	K^+	Cl^-	Hb
TSH	Hypo	Correlation Coefficient	1.000	.405*	.419*	.138	.298	.098	-.495*
		N	100	100	100	100	100	100	100

* Correlation is significant at the 0.05 level (2-tailed).

4.5.2 Correlation between plasma TSH, plasma urea and creatinine, electrolytes and haemoglobin levels in hypothyroid female participants

A significant negative correlation between TSH and Hb levels was observed (r= -0.495). A significant positive correlation was noted between TSH and urea and creatinine (r= 0.405, 0.419). No significant correlation was noted with electrolytes.

Table 7: Euthyroid (Control): Correlation between plasma TSH and parameters

	Group		TSH	Urea	Creatinine	Na^+	K^+	Cl^-	Hb
TSH	Euthyroid	Correlation Coefficient	1.000	.165	.037	.077	.066	.054	.115
		N	174	174	174	174	174	174	174

* Correlation is significant at the 0.05 level (2-tailed).

4.5.3 Correlation between plasma TSH, plasma urea and creatinine, electrolytes and haemoglobin levels in the euthyroid group

On analysis of the values, a negative but insignificant correlation was noted between TSH and Cl^-. No significant correlation was noted between TSH and urea, creatinine, Na^+, K^+ and Hb levels.

CHAPTER FIVE

DISCUSSION

From this study, the levels of plasma urea and creatinine were elevated in females with hyperthyroid and hypothyroid disease compared to participants without thyroid disorder. The findings may be attributed to weight loss because of increased catabolic muscle activity, which also had an early onset compared to impaired glomerular filtration rate in the kidney. The results of the current study agreed with a study carried out by Giordano *et al*., (2001) where he reported increased plasma urea and creatinine in hyperthyroidism and attributed this to increased catabolic activity on muscle and other protein-containing tissue. This was attributed to elevated levels of thyroid hormone that caused an increase in the basal metabolic rate.

Cox and Nelson (2010) pointed out that thyroid hormone increase the basal metabolic rate by acting through nuclear receptors to stimulate energy yielding metabolic pathways in the liver and muscle by increasing the expression of genes encoding catabolic enzymes.

An increase of plasma urea and creatinine in hypothyroid states may be attributed to impaired glomerular filtration, resulting in reduced renal excretion and clearance of urea and creatinine leading to accumulation of plasma creatinine and urea. Manuel, Wiesli, Brandle, Schwegler and Schmid, (2003) also noted an increase in plasma urea and creatinine in hypothyroid states resulting from accumulation due to reduced elimination of protein metabolites by the kidneys.

The current study noted insignificant difference in the plasma levels of electrolytes between females with thyroid disease and those without the disease, Na^+, K^+ and Cl^- ion levels remained within normal reference range. This was probably because female

participants who presented with subclinical type of hyperthyroid and hypothyroid disease did not exhibit extreme levels of thyroid hormone, which would otherwise affect plasma electrolyte levels by interfering with the renal blood flow and glomerular filtration rate, resulting in abnormal clearance. Electrolyte changes are probably experienced in severe forms of thyroid disorders such as myxoedema, which was not evident. The results of this study agreed with a study carried out by Schwarz, Alexander, Spiros and George, (2012) noted low plasma Na^+ in subjects who exhibited hypothyroidism but at high TSH levels. Patients are described as having overt hypothyroidism.

Results from this study showed reduced haemoglobin levels in females with hypothyroidism. A low haemoglobin level was not evident in female participants with hyperthyroidism. Females without thyroid disorder had haemoglobin levels within the normal range. A low haemoglobin level is indicative of anaemia, described as inadequate number of red blood cells or an inadequate amount of haemoglobin in red blood cells.

The current study agreed with a study carried out by Dorgalaleh *et al*., (2013) who noted that in the absence of thyroid hormone anaemia may develop. On the contrary, low levels of haemoglobin have been found to be present in hyperthyroid subjects the cause of which is uncertain and it's often the cause of lethargy in hyperthyroid patients (Hegazi and Ahmed, 2012).

Miłosz, Katarzyna and Edyta (2010) noted that anaemia was not often observed in patients with hyperthyroidism. They also observed that anaemia in hypothyroid patients is not always distinct because it can be complicated by other nutritional deficiencies. Anaemia persists despite therapy with iron, vitamin B12, or folic acid. The degree of anaemia is described as being mild to moderate due to the variation.

The haemoglobin level is rarely less than 8 to 9 g/dl, the normal reference value being 12g/dl (WHO/, CDC, 2008**).**

Tortora and Derrickson (2012) pointed out that 2,3-bisphosphoglycerate (2,3-BPG) an inorganic phosphate produced in red blood cells that decreases the affinity of haemoglobin for O_2 and thus helps unload O_2 from haemoglobin to the tissues. 2, 3-BPG is formed in red blood cells when they break down glucose to produce adenosine triphosphate (ATP) in a process called glycolysis. The greater the level of 2, 3-BPG, the more O_2 is unloaded from haemoglobin making it available to tissues. Thyroxine increases the formation of 2, 3-BPG. Reduced thyroid hormone in hypothyroidism causes anaemia resulting from reduced erythropoietin production by the kidneys. Erythropoietin stimulates the synthesis of red blood cells in the bone marrow a process known as erythropoiesis. Reduced erythropoiesis reduces the formation of red blood cells and haemoglobin, the oxygen carrying pigment and 2, 3-BPG an inorganic phosphate compound that facilitates release of oxygen from haemoglobin to the body's tissue cells. This results in low circulatory Hb levels and reduced availability of oxygen to the body's tissue cells resulting in hypoxia.

In the current study patients having hypothyroid and hyperthyroid disease where classified to into groups depending on the levels of TSH. A significant proportion of patients had subclinical thyroid disease. The presence of subclinical thyroid disorders among the participants confirms the presence of disease despite the presentation of vague or generalized symptoms. Mansourian (2012) noted that overt hypothyroidism is responsible for kidney disorder but observed that sub-clinical hypothyroidism can also interfere with renal function test. This was noted by elevation of creatinine concentration, which is key laboratory test in the diagnosing of kidney disorders can be accompanied with sub-clinical hypothyroidism.

The study correlated TSH with plasma urea and creatinine, electrolytes Na^+, K^+ and Cl^- and haemoglobin levels among the groups. Plasma TSH levels is an indicator for the presence of hyperthyroidism or hypothyroidism. A reduced TSH level below the normal reference range indicates the presence of hyperthyroidism, elevated levels above the normal reference range is an indication of hypothyroidism.

The levels of TSH in hyperthyroid females correlated inversely with increased plasma levels of urea and creatinine. This was probably due to increased protein catabolism and reduced overall muscle bulk despite increased renal clearance caused by increased renal blood flow associated with elevated levels of thyroid hormone. These findings concurred with the study by Ambali (2007) that indicated the presence of hyperureamia and hypercreatineamia in hyperthyroid states. This was probably attributed to imbalance of the equilibrium between renal clearance and the accumulation of protein metabolites. Increased catabolism of proteins in tissues surpassed the rate of renal clearance and lead to accumulation of plasma urea and creatinine.

Elevated TSH levels in the hypothyroid participants correlated directly with an increase in plasma urea and creatinine level. Female participants with hypothyroidism exhibited elevated levels of urea and creatinine. An elevated plasma level of protein metabolites was probably as a result of impaired renal clearance of plasma urea and creatinine. Mansourian (2012) noted that patients with hypothyroidism exhibit a reduction in renal blood flow and glomerular filtration rate (GFR) mainly as result of vasoconstriction. Abdella (2013) noted that hypothyroidism affects tubular function and transport system. Thyroid disorder causes significant changes in kidney function. Hypothyroidism associated kidney disorder seems to be more related directly to a reduction in thyroid hormone levels rather than with thyroid autoimmunity.

In the current study, TSH levels in female participants with thyroid gland disease displayed no correlation with plasma electrolyte levels. Na^{+}, K^{+} and Cl^{-} remained within the normal reference range. This was contrary to a study by Mansourian (2012) who noted reduced plasma electrolyte levels and attributed this to a reduction in the Na^{+}/K^{+} ATPase pump activity leading to the reduction of Na^{+} reabsorption. Reduced thyroid hormone can have a direct effect on reducing renin biosynthesis in the kidney. Renin is an enzyme catalyzing the production of angiotensin-I from angiotensinogen which itself is released from the liver and into the blood circulation. Angiotensin-II in the lung is produced from angiotensin-I catalyzed by angiotensin converting enzyme. Angiotensin-II is the main factor in stimulating adrenal cortex to produce aldosterone the key hormone in the reabsorption of Na^{+} from the kidney. Depressed thyroid hormone reduces the efficiency of the renin–angiotensin system and thus interferes with the reabsorption of Na^{+}. Thyroid hormone reduction interferes with potassium metabolism within the kidney proximal tubules.

Depressed TSH in hyperthyroid female participants had no correlation with haemoglobin levels. The level of haemoglobin remained within the normal reference range. Results suggested that anaemia was not a common feature in patients suffering from hyperthyroidism. Ford (1990) reported that hyperthyroid patients exhibit variations in erythropoiesis, which may lead to haemoglobin levels being high, low or normal. This was attributed to ineffective erythropoiesis due to the presence of reduced iron utilization. On the contrary, results of the current study did not agree with a study carried out by Geetha and Srikrishna, (2012) who reported the significant presence of anaemia in hyperthyroid female patients.

Findings from this study showed an inverse correlation between elevated TSH levels with reduced levels of haemoglobin in hypothyroid patients. This was attributed to

reduced stimulation in secretion of renal erythropoietin hormone. Yaluan *et al*., (2004) noted that thyroid hormone has been found to increase the oxygen capacity of blood and to improve perfusion by vasodilation of blood vessels by increasing the production and secretion of the hormone erythropoietin (EPO) in the kidney. The mechanism underlying these actions remains unclear. Suppression of thyroid hormone reduces this function resulting in diminished production of EPO and subsequently reduced hyperplasia of erythroid progenitors in the stem cell resulting in reduced erythrocyte and haemoglobin production.

In the study, female participants confirmed with thyroid disorder displayed the existence of biochemical derangement following analysis of associated haemoparameters. The findings of the present study indicated that females of reproductive age with thyroid gland disease exhibited some significant clinical manifestations which are often overlooked due to the their subtle presentation that often go unnoticed as signs and symptoms that could be associated with thyroid gland disorder.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The findings from this study leads to the rejection of the null hypothesis and the acceptance of the alternate hypothesis.

In conclusion, the findings of the present study showed that thyroid gland disorder comprising hypothyroidism and hyperthyroidism in females of childbearing age is accompanied by derangement in some haemoparameters. This study showed that

1. There was abnormal elevation in plasma urea and creatinine levels in female patients with both hypothyroidism and hyperthyroidism.
2. There was a normal level of plasma electrolyte Na^+, K^+ and Cl^- in female patients with thyroid disorder.
3. There was abnormal decline in the levels of haemoglobin in female patients with thyroid gland disorder. The decline was evident in the hypothyroid participants.
4. There was presence of subclinical hypothyroidism and hyperthyroidism in females patients with thyroid disorder.

6.2 RECOMMENDATION

From the findings of this study, it is recommended that routine screening and testing for TSH levels should be carried out in:

1. Women diagnosed with unexplained chronic anaemia confirmed by persistently depressed haemoglobin levels.
2. Patients should be advised by doctors to have their thyroid function checked routinely.

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APPENDICES

APPENDIX I

IREC LETTER

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2013/40
Approval Number: 0001042

26th August, 2013

Ms. Khadija Nyagaka,
University of Eldoret,
School of Biological Sciences,
P.O. Box 1125-30100,
ELDORET-KENYA.

Dear Ms. Khadija,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee have reviewed your research proposal titled:-

"Evaluation of Haemoparameters and Thyroid Hormone Levels in Female Patients with Thyroid Gland Disorders at M.T.R.H".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1042*** on 26th August, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 25th August, 2014. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
26 AUG 2013
P.O. Box 4606 ELDORET

Sincerely,

05/09/2013
DR. W. ARUASA
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	Director-	MTRH	Dean -	SOM	Dean -	SON
	Principal-	CHS	Dean -	SPH	Dean .-	SOD

APPENDIX II

Materials

Test Kit from Pishtaz Teb Diagnostics

96 tests kit

Enzyme Immunoassay for quantitative determination of concentration in human plasma of TSH, free $T_{3,}$ and T_4 respectively.

Equipment

BioTek® EL x50 Automatic microplate strip washer

BioTek® spectrophotometer ELISA reader

Precision pipette

Distilled water

Disposable pipette tips

Absorbent paper /gauze

Centrifuge

APPENDIX III

Normal values for haemoglobin, urea, creatinine, electrolytes (Na^+, K^+, and Cl^-) and thyroid hormone levels

Normal values for haemoglobin (Hb) levels in non-pregnant females (WHO/, CDC, 2008)

Test item	Unit	Ref. Range
Hb	g/L	12-16.5

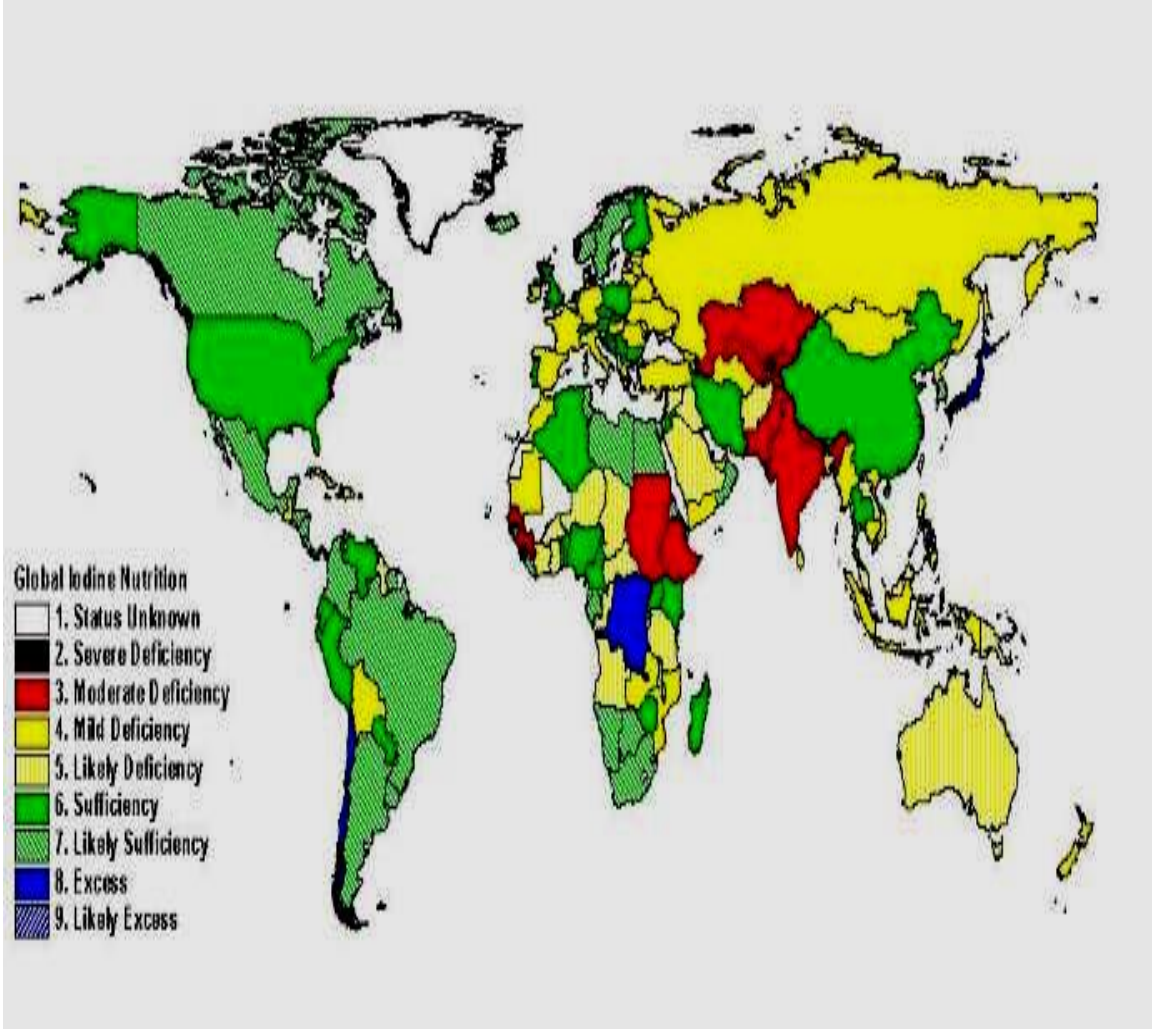
Normal values for plasma urea, creatinine, sodium, potassium, and chloride ions (Fauci, 2008)

Urea	Creatinine	Sodium (Na^+)	Potassium (K^+)	Chloride (Cl^-)
2.5-7 mmol/L	44-80 umol/L	135-148 mmol/L	3.5-5.1 mmol/L	97-108 mmol/L

Normal values for thyroid hormone levels (Pishtaz Teb Diagnostics MA-TSH-96-01, MA- FT3, 96-01, MA-FT4, 96-01)

TSH (mIU/L)	FT_3 (ng/ml)	FT_4(ng/dl)
0.32-5.2	0.6-2.1	0.7-1.8

APPENDIX IV



Map 1: World's Iodine Nutrition (Source: Fauci, A.S., and Kasper, D. L. *Harrison's Principles of Internal Medicine.* 17th ed. http: //www.accessmedicine.com)

APPENDIX V

Map 2: Geographical location of Moi Teaching and Referral Hospital Uasin Gishu County Kenya

APPENDIX VI

INFORMED CONSENT FORM

"Evaluation of Haemoparameters and Thyroid Hormone Levels in Female Patients with Thyroid Gland Disorders."

You are invited to participate in a research study conducted by MPhil candidate from the University of Eldoret. This study is being conducted at the Moi Teaching and Referral Hospital.

You must be 18 years or older to participate in the study. Your participation is voluntary.

The research will involve drawing a minimal amount of blood using a syringe and needle and collecting it in a tube, the extracted blood will be sent to the Moi Teaching and Referral Hospital laboratories for investigations. There are no major risks to your participation. Minimal anticipated risks will include pain at the site of injection. You will not directly benefit from your participation in this research.

The information given is confidential and strictly for research. Your identity will be coded in order to protect your confidentiality.

The purpose of the study has been explained to me and I have fully understood the procedures to be used, the benefits, risks, hazards and discomforts associated with the procedures to be undertaken.

The investigator my take blood samples from me for the tests required for the study. I understand that I may at any time during the course of this study, revoke my consent and withdraw from the study without any penalty.

If you wish to be in this study, please tick yes or no in the boxes provided.

Yes ☐ No ☐

APPENDIX VII

FOMU YA KUTOA IDHINI

MIMI Bw. /Bi.……natoa idhini

Kwa…….....Kunijumuisha katika utafiti unaopendekezwa kuhusu:

“Ukadirifu wa Vigezo vya ‘Hemo’ na Viwango vya Homoni za Tezi katika Wagonjwa wa Kike walio na Matatizo ya Glandi za Tezi.”

Nimeelezwa matilaba/kusudi la utafiti huu na nimeelewa kikamilifu taratibu zitakazofuatwa, faida, hatari na uchechefu unaohusishwa na taratibu zitakazotumika.

Nakubali kwamba Mchunguzi anaweza kuchukuwa sampuli ya damu yangu kwa uchunguzi unaohitajika katika utafiti huu. Naelewa kuwa nina uwezo wa kubatilisha idhini yangu wakati wowote ule kabla ya kukamilishwa kwa utafiti huu bila ya kuadhibiwa au kunyimwa manufaa ya kimatibabu.

Hautalipwa kwa kujumuishwa katika utafiti huu wala hakutakuwa na gharama yeyote kwako kwa sampuli za damu zitakazokusanywa na kuchunguzwa. Ni hiari yako kujumuishwa katika utafiti huu.

☐ Ndio ☐ LA

Plate 2: BioTek EL x50 Automatic microplate strip washer 12 well (USA) (Source: Author, 2014)

Plate 3: ABBOTT Commander Incubator (USA)
(Source: Author, 2014)

Plate 4: BioTek® spectrophotometer ELISA reader (USA)
(Source: Author, 2014)