CHARACTERIZATION OF THYROID DISORDERS AMONG PATIENTS ATTENDING SURGICAL CLINIC AT NAKURU LEVEL 5 HOSPITAL - NAKURU COUNTY, KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL BIOCHEMISTRY, SCHOOL OF MEDICINE, MOI UNIVERSITY,

DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for the award of a degree in any other University. No part of this thesis may be reproduced or transmitted in any form without the prior written permission of the author and / or Moi University.

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DEDICATION

I dedicate the accomplishment of this thesis to my great family friend, the late Joel Kipruto Chepkwony, my family and my professional colleagues.

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ABBREVIATIONS

(NIS)	Na+/I-symporter
(RXR)	Retinoid X Receptor
5'-D	5'-deiodinase
9-CIS RA	denotes 9-cis-retinoic acid, the ligand for RXR
AITD	Auto-immune Thyroid Disease
CA	Cancer
cAMP	Cyclic Adenosine Mono-phosphate
D1	Type 1 deiodinase
D2	Type 2 deiodinase
D3	Type 3 deiodinase
DIT	Di-iodotyrosine
DNA	Deoxyribonucleic acid
FGF 23	Fibroblast growth factor-23
FNAC	Fine Needle Aspirate for Cytology
HLA	Human Lymphocytic Antigen
HPT	Hypothalamic/pituitary/thyroid gland axis
HRC	Hormone-Receptor Complex
HRNT	Hormone-responsive nuclear transcription factors
IDD	Iodine Deficiency Disorders
IFNγ	Gamma Interferon
IL	Interleukin
MHC	Major Histocompatibility Complex
MIT	Mono-iodotyrosine
NCG	Nodular colloid Goitre
RAI	Radio – Active Iodine

RAIU	Radio – Active Iodine Uptake
RNA	Ribonucleic acid
STN	Solitary Thyroid nodule
T_3	Tri-iodothyronine
T_4	Tetra-iodothyronine (Thyroxine)
TBG	Thyroxin Binding Globulin
TFT	Thyroid function Test
Tg	Thyroglobulin
THRE	Thyroid hormone response elements
TNF	Tumour Necrosis Factor
TPO	Thyroid Peroxidase
TR	Thyroid Receptor
TRH	Thyrothropin-releasing hormone
TSH	Thyroid Stimulating Hormone
TSHR	Thyroid Stimulating Hormone Receptor
TSHRAb	Thyroid Stimulating Hormone Receptor Antibodies
TSI	Thyroid Stimulating Immunoglobulin
VAD	Vitamin A deficiency
WHO	World Health Organization

DEFINITIONS

Thyroid disorders: This refers to a diversity of diseases due to abnormal 'function' and / or 'structure' of the thyroid gland.

Hyperthyroidism: An overactive thyroid gland leading to elevated thyroid hormones

Hypothyroidism: Is a condition of decrease thyroid hormones function.

Euthyroidism: Is a state of normal thyroid hormone function in the body

Sub-clinical Hyperthyroidism: This refers to a condition of no clinical signs and symptoms of thyroid dysfunction with normal thyroid hormones (T_4 & T_3) but suppressed TSH.

Sub-clinical Hypothyroidism: This refers to a condition where there is elevated TSH and normal thyroid hormones (T_4 & T_3) but the patient is clinically asymptomatic.

Overt Hyperthyroidism (hyperthyroidism): Characterized by a decreased TSH and an increased T_4 level.

Overt thyrotoxicosis: Is characterized by high serum free T_4 and T_3 and very low serum TSH

Overt Hypothyroidism (hypothyroidism): Characterized by an increased TSH and a decreased T_4 level.

Type 1 & type 2 deiodinase (D1 and D2): Convert T_4 into T_3

Type 3 deiodinase (D3): Degrades T_4 and T_3 into inactive metabolites.

Consumptive hypothyroidism syndrome (CHS): Is an uncommon and severe form of hypothyroidism that occurs due to high levels of D3 expression by neoplastic tissues, resulting in high rates of thyroid hormone inactivation.

Immuno-assay: Is a technique or test used to detect the presence or quantity of a substance (as a protein) based on its capacity to act as an antigen or antibody.

Goitre: An abnormal swelling of the neck due to enlargement of the thyroid gland. Occurs when the thyroid gland is unable to meet the metabolic demands of the body with sufficient hormone production and the gland compensate by enlarging.

Colloid goitre: Are benign, non-cancerous enlargement of an otherwise normal thyroid gland and does not spread beyond the thyroid gland and can either be diffuse or nodular

Nodular Goitre: Where solid or fluid-filled lumps called nodules develop within the thyroid and make the thyroid gland feel lumpy to touch; the nodules can be single or multiple.

Diffuse Goitre: Where the entire thyroid gland swells and feels smooth to the touch.

Euthyroid / Simple Goitre: Thyroid enlargement not accompanied by constitutional effects e.g. inflammation, hypothyroidism or hyperthyroidism, commonly caused by inadequate dietary intake of iodine.

Endemic Goitre: Goitre occurring widely in a geographical region where food or water is deficient in iodine. An area is defined as endemic for goitre, if more than 10% of children aged 6 – 12 years have goitre.

Goitrogens: Substances that disrupt the production of thyroid hormones by interfering with iodine uptake in the thyroid gland.

Hashimoto's disease: Also known as chronic lymphocytic thyroiditis. Is a progressive disease of the thyroid gland characterized by the presence of antibodies directed against the thyroid and by infiltration of the thyroid gland by lymphocytes (WBC's activated by immune system)

Graves' disease: Swelling of the neck and protrusion of the eyes resulting from overactive thyroid gland.

Fibroblast growth factor-23: Is a potent regulator of phosphate and vitamin D homeostasis

Response element: A specific sequence of DNA that a transcription factor (HRC) binds to.

The human leukocyte antigen (HLA) system [MHC]: Is an important part of the immune system and is controlled by genes located on chromosome 6 and encodes cell surface molecules specialized to present antigenic peptides to the T-cell receptor (TCR) on T cells

Enhancers: Contain DNA sequences called response elements (RE) that binds specific transcription factors called 'Activators'

Motifs: Are structures e.g. Zinc fingers, leucine zippers and helix-turn-helix through which DNA is bound by proteins.

Activators: Are transcription factors / DNA-binding proteins that bind to enhancers or promoter region of DNA and increases gene transcription or set of genes

Transcription factor: Is a protein that binds to DNA and regulate gene expression by promoting or suppressing transcription

Gene expression: the process by which information from a gene is used in the synthesis of a functional gene product such as a protein

Transcription: the process of making messenger RNA (mRNA) from a DNA template by RNA polymerase II

Transcription regulation: Controlling the rate of gene transcription / expression for example by helping or hindering RNA polymerase II binding to DNA.

Co- activator: A protein that works with transcription factors to increase the rate of gene transcription

Co-repressor: A protein that works with transcription factors to decrease the rate of gene transcription

Up regulation: Activation or promotion –increasing the rate of gene transcription.

Down-regulation: Repression, or suppression –decrease the rate of gene transcription.

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ABSTRACT

Background: Thyroid disorders are among the most common endocrine disorders seen worldwide. Among these disorders, goitre is commonest in Kenya. Thyroid disorders are manifested by derangement of thyroid hormone secretion, goitre formation and / or pain. Thyroid disorders severely affect the quality of life as they usually result in compressive symptoms, impaired physical and intellectual development and pregnancy complications. The present study sought to characterize thyroid disorders to avail baseline data that can be relied upon for preventive policy development, improvement of patient treatment, care and management for better disease prognosis. Differential diagnosis for thyroid disorders is performed by biochemical assays, ultrasound, and fine needle aspirate.

Objectives: To identify socio-demographic factors associated with biochemical and non-biochemical characteristics of thyroid disorders and common clinical presentation among patients attending surgical clinic at Nakuru level 5 Hospital.

Methods: A descriptive, cross sectional study was conducted from 4th February to 28th June 2018 at Nakuru level 5 Hospital surgical clinic using structured questionnaires. Sampling was done by census enrollment of patients with thyroid disorders or clinical features of thyroid disorders with or without goitre and who fulfilled the eligibility criteria. Patients' clinical findings and demographic characteristics were documented. Thyroid hormonal profile and auto-antibodies were performed using automated Electrochemiluminescence-Immuno-assay method. Serum iodide levels estimation was performed using Iodometric titration techniques. Other investigation techniques including thyroid ultrasound and Fine needle aspirate for cytology were also done for 123 and 15 participants respectively.

Results: The mean age of participants was 40.5 years (SD: 15.4) comprising 114 (92%) females and 10 (8%) males. Majority 102 (82.3%), were in the age group 21- 60 years, with females representing 90.2%. The most common clinical presentation was goitre 95 (76.6%). Based on biochemical assays (T_4, T_3 and TSH), 66 (55%) were Euthyroid, 48 (40.0%) hyperthyroid, 4 (3%) subclinical hyperthyroid and 1 (1%) each for both hypothyroid and subclinical hypothyroid. Thyroid ultrasound detected 86 nodular goitres, 27 diffuse goitres, 7 thyroid masses and 3 solitary thyroid nodules. Thyroid stimulating immunoglobulin antibodies (diagnostic of Graves' disease) were detected in 19 (39.6 %) of hyperthyroid patients. Serum iodide levels (Iodometric titration) revealed 48 (40%) patients with slightly elevated iodide levels >18 µg/dl, 1 (1%) had low iodide level < 5.0 µg/dl, while 71 (59%) had iodide levels within normal ranges (5 - 18µg/dl). Toxic nodular goitre (based on ultrasound, raised T_4 and decreased TSH) were found in 26 (54.2%) of hyperthyroid patients and was the commonest pathological cause of hyperthyroidism, followed by Grave's disease. Fine needle aspirate for cytology (FNAC) was conducted in 15 (12.1%) suspected cases of malignancy, with 7 (46.7%) being confirmed histologically as malignant goitres.

Conclusion: Goitre was the most common presentation of thyroid disorder with toxic nodular goitre contributing 54.2% of the hyperthyroid patients and was most common in females. The peak age of presentation was 21 – 60 years.

Further studies are required to elucidate the risk factors contributing to the increase in cases of goitres especially among females in the central rift valley region of Kenya.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Thyroid disorders are medical conditions that affect the 'functions' and / or 'structure' of the thyroid gland, an endocrine organ that synthesizes and secretes thyroxine (T_4) and tri-iodothyronine (T_3) both of which are hormones essential for normal metabolism in many animal species including man. The principal extrinsic ingredient of these hormones, iodine, is avidly taken up by the thyroid gland by a process which results in the thyroid gland maintaining concentration gradients for iodine against plasma levels reaching 20 - 50 foId (Chung et al. 2014).

Iodine (number, 53; standard atomic mass, 126.9) is a rate-limiting element for the synthesis of thyroid hormones and at present, the only physiological role known for iodine in the human body is in the synthesis of thyroid hormones by the thyroid gland (Chung et al. 2014).

Thyroid disorders are among the most common endocrine disorders seen worldwide and the second most common disorders after diabetes mellitus (Ogbera et al. 2011). Thyroid disorders are manifested by derangement of thyroid hormone secretion, goitre formation and / or pain. Thyroid function is crucial to the metabolism of almost all tissues and is critical for the development of the central nervous system in the fetus and children (de Escobar et al. 2007). The relationship between iodine deficiency and thyroid disorders / disease was known since early in the twentieth century. Iodine deficiency has been regarded as one of the most important preventable causes of brain damage worldwide (Zimmermann et al. 2009). In 2013, 30 countries remain iodine-deficient; 9 are moderately deficient, and 21 are mildly deficient, defined by median urinary iodine (UI) in school-aged children (Pearce et al. 2013). While the prevalence

of severe iodine deficiency was reduced recently, the problems of iodine some deficiency re-emerged in vulnerable populations, such as pregnant women and infants. Furthermore, some food or medications have very high iodine contents, which can result in thyroid dysfunctions in some susceptible individuals (Chung et al. 2014).

The term Iodine deficiency disorders (IDD) covers a wide spectrum of disorders including goitre and cretinism and these conditions are prevalent in several developing countries, Kenya included. The symptoms of thyroid disorders vary depending on the type (Caturegli et al. 2014). The thyroid gland is an important endocrine gland anatomically located inferior to the larynx and anterior to trachea (Braverman et al. 2012). In health, this gland secretes thyroxine, (T_4) and tri-iodothyronine, (T_3) hormones in the right amounts which regulate the metabolic activities in the human body and whenever there is instability in the amounts of these two hormones, undesired pathologic effects occur which are termed as diseases / disorders, of the thyroid, which can either affect the 'structure' and / or the 'functions' of the thyroid gland (Mansourian et al. 2011). The population at risk to thyroid disorders includes the elderly, adolescents, neonates, women of reproductive age and in pregnancy and also in women at menopause (Ogbera et al. 2011). Genetic, nutritional, environmental factors and the presence of other immunologic based disease such as Hashimotos and Graves' disease contribute to the high prevalence of thyroid disorders. Other risk factors that aggravates the prevalence of thyroid disorders include exposure of the neck or head to harmful radiations, hypothalamic and pituitary abnormalities, drug therapies such as amiadorone, serious chronic illness such as thyroid tuberculosis and majorly iodine deficiency (Zosin et al. 2012).

Thyroid disorders are one of the most common endocrine disorders seen world-wide and are endemic in many parts of the world and much of this is related to Iodine

deficiency disorders (IDD) which top the list of thyroid disorders and remains the commonest cause of thyroid disorders in the African continent and is affected not only by the iodine status in the region but also by selenium deficiency and thiocyanate toxicity (Ogbera et al. 2011).

Iodine deficiency disorders contribute to complications in physical and intellectual development that can lead to cretinism in severe cases during childhood, and pregnancy complications (Ogbera et al. 2011). It is estimated that over 750 million people worldwide are affected by IDD, and with goitre, a major thyroid disorder caused by iodine deficiency is approximated to be as high as 80% in some endemic areas (Vanderpump et al. 2011). These areas tend to be remote and mountainous. Such good example of topographically affected areas include the south-east Asia, Latin America and central Africa where there is high morbidity of iodine deficiency disorders. The WHO recommended the fortification of foods, especially salt, by food processing companies as a measure to curb Iodine deficiency disorder (IDD) and ideal dietary allowance of iodine intake is 150 µg of iodine per day and should be increased to 250 µg in pregnancy and 290 µg in lactation is required to maintain a physiological plasma iodide concentration of approximately 5 – 18 µg / dl (Organization et al. 2014). The dietary programme proved to be of great value in reducing goitre sizes and prevented goitre development in the key population and cretinism in children. However, WHO still estimates that 2.2 billion people of which 285 million are of school age children still have iodine deficiency defined by urinary iodine excretion of less than 100 µg/L. It was later discovered that the iodization programme could also lead to thyrotoxicosis especially to those aged 40 years and above and having nodular goitre (Zimmermann et al. 2009). A rising concern is that the population with thyroid disorders in iodine-replete areas have been found to have autoimmune thyroid disease

ranging from primary atrophic hypothyroidism, Hashimoto's thyroiditis to thyrotoxicosis as a result of Grave's disease. Such cases of autoimmune thyroid diseases are under-diagnosed especially in developing and under-developed countries The most common thyroid disease was simple physiological goitre in which ultrasonography imaging has been used in epidemiological studies to assess the thyroid size and physical examination which greatly reduced many cases of goitre (Vanderpump et al. 2011). In Athens Greece autoimmune thyroid disease was shown to be the most common cause of thyroid disorder among children and adolescents and has a wide spectrum of clinical manifestations and a variable clinical course (Skarpa et al. 2011). It was estimated that the prevalence of positive thyroid auto antibodies was found to be between 4-10%, goitre 3.2%, thyroid nodules 5.1% and altered thyroid echo structure approximately 9%. A decade after the elimination of iodine deficiency in Greece, it has been noted there has been an increase in the prevalence of autoimmune thyroiditis (Skarpa et al. 2011). In African continent, dietary iodine deficiency is the major determinant of thyroid pathology resulting in a spectrum of iodine deficiency disorders including goitre, hypothyroidism and mental retardation and cretinism with mental retardation having been shown to pose the most severe threat to socio-economic wellbeing (Organization et al. 2014). It is estimates that 43% of the population in the African region, or approximately 260 million people, have insufficient iodine intake (Saha et al. 2019). In Kenya it is estimated that 20% of the households suffer from iodine deficiency disorders, after a systematic review to evaluate iodine status in women of reproductive age and pregnant women (Harika et al. 2017). A follow-up study designed to determine the prevalence of IDD by measuring urinary iodine excretion among primary school age children, showed that IDD prevalence in Nairobi was 3.5%, which is within the WHO value of less than 5%

suggesting that the study community (school children) is iodine sufficient (Kishoyian et al. 2014). World Health Organization has put in place universal salt iodinization as the primary strategy to address the morbidity from iodine deficiency, particularly the neurologic consequences (Zimmermann et al. 2009). It is also estimated that endemic goitre due to iodine deficiency is present in 28.3% of the African population compared to 25% globally. Efforts of eliminating iodine deficiency in Africa has been frustrated by civil conflicts, fragile political structures and poverty (Assey et al. 2009).

Goitre which is more frequent in females than males, is the most common manifestation of thyroid disorder in many parts of the world and occurs in 3% to 5% of the world's population (Vanderpump et al. 2011). Nitrates are among the major inorganic pollutants in the environment primarily contributed by nitrogenous fertilizers, organic manures, humans and animals' wastes and industrial effluents through biochemical activities of micro-organisms. Beside other known detrimental effects, research findings suggest that increased ingestion of nitrates in water at high doses can competitively inhibit iodine uptake by the thyroid gland and induces hypertrophy of the thyroid gland thus affecting its functions in humans (De Groef et al. 2006). In Kenya goitre remains endemic despite iodization of table salts in households, notably, the area around Nandi hills due to high concentration of nitrate ions in drinking water as a result of application of inorganic fertilizers and animal manure in agricultural areas (Tarus- Sharon et al. 2018). Areas which have endemic goitre in Kenya are central and western highlands parts of Rift Valley and most of the cases of goitre from these areas do not show iodide deficiency on biochemical evaluation. Many of these patients manifest clinical and laboratory features of simple / Euthyroid goitre (normal plasma levels of thyroxine, tri-iodothyronine, thyroid

stimulating hormone and normal thyroid iodide uptake values). When dietary iodide levels are inadequate to maintain blood level because of either absolute or relative deficiency, physiological feedback mechanisms result in enlargement of the thyroid gland (goitre) in an attempt to enhance its iodide-trapping activity. Endemic goitre (when the prevalence of goitre exceeds 10 percent in a given population) is characterized by enlargement of the thyroid gland in a significantly large fraction of a population group, is a major feature of Iodine deficiency disorders (IDD) and is by far the most commonly observed thyroid disorder and continues to be a problem in many African countries (Ogbera et al. 2011).

The majority of affected people live within subsistence agricultural systems, commonly in mountainous regions which have been deprived of iodine by leaching of the soil. Goitrogens in local foods like cassava and millet accentuate the effects of iodine deficiency (Kazungu et al. 2018) and other micronutrient deficiencies such as selenium also play a role in the development of goitre. The prevalence of goitres varies widely but may be as high as 85% in children in some areas (Zimmermann et al. 2012). Recent improvements in iodine intake have led to reductions in goitre prevalence but goitres prevail in communities who continue to rely exclusively on home grown crops for nutrition. The prevalence rates of endemic goitre in Africa range from 1% to 90%. Other manifestations of iodine deficiency include, endemic cretinism and development of hyperthyroidism in multinodular goitre, the latter is not widely studied in the African continent (Ogbera et al. 2011). Children, females and pregnant women remain disproportionately affected. Recent studies have shown that total goitre rates of close to a 100% in pregnant women, even in areas within countries with iodine sufficiency status (Andersson et al. 2010) It is important to note that although there is a demonstrable association between iodine deficiency and endemic

goitre, goitrogens (substances which inhibit iodide transport mechanisms within the thyroid gland) may also play a role in the development of endemic goitre. Goitrogens include in-organic anions, notably perchlorate and thiocyanate that are often found in poorly detoxified cassava, a staple food that is commonly eaten as a source of carbohydrate. Enzyme-dependent hereditary biosynthetic defects are a rare cause of goitre. Selenium deficiency has also been reported to be a contributory factor in the occurrence of endemic goitre in Africa or persistence of endemic goitre in iodine deficient areas even after correcting for iodine deficiency (Kishosha et al. 2011). The extreme form of iodine deficiency, endemic cretinism has been well characterized in Central Africa, where up to 2–6% of the overall population may be affected. The myxedematous form of cretinism is highly prevalent in this population and hypothyroidism is seen in as much as a quarter of children in endemic areas (Chen et al. 2010). This pattern contrasts with other parts of the world where the neurological variety, characterized by mental deficiency, deaf mutism and spastic diplegia prevails. However, gross neurological defects are also seen in African populations and were described in 10% of patients with cretinism in central Africa (Spencer et al. 2012).

Less obvious disorders of cognition and intellect in infancy and childhood are likely to be even more widespread and to potentially constitute a greater burden on the economic output of affected communities (Melse-Boonstra et al. 2010). Thyrotoxicosis may occur in chronically iodine deficient individuals who are exposed to sharp increases in iodine intake (Sun et al. 2014). It is more likely in older patients with longstanding thyroid nodules. Toxic change in such nodules may be accompanied by biochemical or clinically overt thyrotoxicosis. Death from cardiac failure and arrhythmias may occur (Sun et al. 2014). Between 1991 and 1995, a sudden rise in the incidence of thyrotoxicosis was noted by physicians in Harare,

Zimbabwe. The majority of these patients had toxic nodular goitres and deaths were recorded. Likewise, biochemical thyrotoxicosis was diagnosed in patients with goitres in Kivu, a previously iodine deficient area of Northern Zaire. These incidents followed the introduction of iodized salt to both countries. Studies have shown that despite public Health measures, such as fortification of salt with iodine, endemic goitre continues to be a major problem. Based on a cross-sectional study published in 2002, 64% of Ugandan households were thought to have adequate iodine intake, but rates of goitres in school-aged children were still quite high at 60%, suggesting that iodine availability is a persistent problem and thus the burden of thyroid disorders is a significant and major public Health concern in this region (Fualal et al. 2012). Data on autoimmune thyroid disorders in Africa is scarce due to under-diagnosis and underreporting. Studies have indicated that 1.2% to 9.9% of thyroid disorders in Africa are due to auto-immunity of which Graves' disease is the commonest (Gebreyohannes et al. 2019). Graves' disease occurs in about 0.5% of the population and is the commonest cause of hyperthyroidism in iodine-replete parts of the world, in developed countries and is now more frequently reported across the African continent (Vanderpump et al. 2011). Incidence of autoimmune thyroid disease due to Graves' disease is currently higher than in historic series although the studies are so variable in design, patient population, disease definition, and laboratory methods. The clinical presentation of Graves' disease appears similar in other areas and females are more commonly affected (McLeod et al. 2012). Opthalmopathy is common and thyroid stimulating hormone-receptor antibodies are present in over 80% of patients (Menconi et al. 2014). Of the autoimmune diseases of the thyroid, Graves' disease is the predominantly documented features of autoimmune disease in Africa (Ogbera et al. 2011). Among the causes of spontaneous thyrotoxicosis, Graves' disease is the most

common. Thyrotoxicosis is the predominant presenting features of Graves' disease, and in this region (Africa) the presenting features of thyrotoxicosis are characterized by the complications of the disease condition. Among patients with thyrotoxicosis, 60% - 80% have Graves' disease, depending on regional factors. The annual incidence in women over 20 years of age is 0.5 per 1000 with the highest risk of onset between the ages of 30 – 60 years but can begin at any age. Graves' disease is the most prevalent auto-immune disorder and the leading cause of hyperthyroidism (50% - 80%) of cases in the United State and develops in about 0.5% of male and 3% of female and is unusual in children (Burch et al. 2015). The prevalence of Graves' disease is similar among whites and Asians but lower among blacks. Blood tests show raised T_4 and T_3, low TSH and presence of Thyroid stimulating immunoglobulins (TSI), that as similar effect to TSH. TSI antibodies cause the thyroid gland to produce excess thyroid hormones. The cause of Graves' disease is unknown, however, it is believed to involve a combination of genetic and environmental factors (Menconi et al. 2014). Those with other autoimmune diseases such as type I diabetes, Rheumatoid arthritis are more likely to be affected more. Smoking increases the risk of disease and may worsen eye problems. The diagnosis may be suspected based on symptoms and confirmed with blood tests and RAIU (Menconi et al. 2014).

The clinical presentation of thyroid cancer is usually as a solitary thyroid nodule or increasing goitre size. Although thyroid nodules are common, thyroid cancers are rare. Thyroid cancer is the most common endocrine malignant tumour and accounts for 90% of the cancers of the endocrine glands. Thyroid cancer is the fastest accelerating malignancy and constituting 2.49% in males and 2.34% in females of all cancers registered in the UK (Smittenaar et al. 2016). Differentiated and undifferentiated thyroid carcinomas are relatively rare, constituted 0.5% to 1% of all

cancer worldwide. Long standing goitre is regarded as one of the most frequent risk factor for the development of follicular thyroid cancer (Pellegriti et al. 2013). Studies show that differentiated thyroid malignancies (Papillary and follicular CA) in Africa are noted to occur more commonly than the other forms of thyroid CA and are among the most curable cancers. Papillary and follicular cancers are rare in children and adolescents, and their incidence increases with age in adults. The median age at diagnosis is 45 to 50 years (Memon et al. 2010). Papillary carcinoma is the most common thyroid cancer followed by follicular, medullary and anaplastic carcinoma. Marked variation in the prevalence of thyroid carcinomas has been observed in different regions of the world (Kumar et al. 2014). Thyroid carcinomas are two to four times as frequent in women as in men and it is more common in the third, fourth and fifth decades of life (Memon et al. 2010). The documented prevalence rates of thyroid CA in the African continent are as follows (papillary: 6.7–72.1%, follicular: 4.9–68%, anaplastic: 5–21.4%, and medullary: 2.6%–13.8%). For the differentiated thyroid CA, there appears changing trend in the more common papillary CA to the rare follicular CA ratio and this may be attributable to widespread iodization programme (Ogbera et al. 2011).

Solitary thyroid nodules are common clinical findings and are mostly benign. Most of the thyroid nodules are due to cystic degeneration change in nodular goitre or colloid cyst while a few of the solitary nodules are neoplastic. The reported prevalence of nodular thyroid disease depends on the population studied and the methods used to detect nodules (Vanderpump et al. 2011). Although the majority of these are benign, a significant number are malignant and prognosis depends upon timely and appropriate investigation and management (Rao et al. 2013). Nodule incidence increases with age, women, in people with iodine deficiency, and after radiation exposure. The

prevalence of single thyroid nodules is 3% and multinodular goitre is 1% (Pellegriti et al. 2013). Ultrasonography imaging is the most accurate and cost-effective method for evaluating and observing thyroid nodules (Cooper et al. 2009).

In Kenya thyroid disorders, among them goitre which is caused by iodine deficiency is prevalent and common in the highland areas of Rift Valley region where the land is fertile and majority of the province's population is concentrated (Davies et al. 2008). The principal control strategy for IDD in Kenya is the fortification of table salt and this iodine replacement programme does not provide the solution in every case, therefore necessitating the study to ascertain factors associated to this scenario especially the competitive and suppressive action of goitrogenic substances with iodine and the body inability to utilize effectively the ingested iodine. The distribution of goitre, in certain areas of Rift Valley, Kenya, is reported at 15-72% and estimates based on assessment of iodine status in the population indicates that 62.8% of the household population of Kenya is at risk of IDD (Harika et al. 2017). There is an existence of a highly significant correlation between total goitre rates and low urinary iodine excretion in the highlands east and west of the Rift Valley, Kenya (Davies et al. 2008) . Studies have indicated that iodine prophylaxis is ineffective in reversing all types of goitre because not all goitres in Kenya are the direct result of iodine deficiency and there is a strong positive linear correlation between the presence of the goitrogens thiocyanate (SCN-) found in starch, one of the ore processing reagents for mining and the existence of endemic goitre rates along KerioValley region in Rift Valley, Kenya (Davies et al. 2008). Goitrogens that may directly or indirectly account for some of the goitre cases in Kenya include the univalent complex ions, thiocyanate (SCN~) found in cassava, kale and the fluo- borate ion (BF_4~), whose ionic sizes are similar to that of iodide. Rocks, soils and natural waters in the Rift Valley areas of

Kenya tend to be high in fluorine as a result of the hydrogen fluoride volcanism associated with the formation of the Rift System (Ozsvath et al. 2009). Studies have shown that there is considerable scientific relationship between high fluoride intake common in the Rift Valley areas and endemic goitre due to competitive inhibition of the active transport of iodide both in the thyroid gland and in extra-thyroidal iodide-concentrating in tissues leading to reduce availability of substrate for thyroid hormones formation (Ozsvath et al. 2009). Concurrent occurrence of fluorosis and endemic goitre may have a causal relationship. Thus the endemicity of goitre in highlands region of Rift Valley, Kenya could be a consequence of relative iodide deficiency in these regions where there is a simultaneously relative abundance of fluoride. In Kenya, iodine sufficiency may have been achieved, but it is not clear why the goitre rate is still high despite 95% household coverage (Zimmermann et al. 2008). It has been suggested it could be due to isolated cases of iodine deficiency, cancer or goitrogens especially thiocyanate ingestion, selenium deficiency and vitamin A deficiency (VAD causes thyroid hypertrophy, reduces thyroidal iodine uptake and impairs synthesis of thyroglobulin). Excess iodine may also be the cause of reactive goitres. It is with this reason that this study was set up to characterize thyroid disorders among patients attending surgical clinic at Nakuru level 5 Hospital base on biochemical and non-biochemical approaches.

1.2 Problem statement

Iodine deficiency disorder (IDD) is a major public Health problem throughout Africa and is the commonest cause of thyroid disorders in the continent. Worldwide, 2.2 billion people are at risk of IDD, of this 30-70% have goitre and 1-10% have cretinism. (Kishosha et al. 2011). It is estimated that 8% of newborn children from sub-Sahara Africa are unprotected from learning disabilities as a result of IDD related

disorders (Organization et al. 2014). Data on auto-immune thyroid disorders in Africa is scarce, thus contributing to problems of under-diagnosis and under-reporting of IDD (Gebreyohannes et al. 2019). Studies have indicated that the prevalence rate of thyroid disorders due to auto-immunity is 1.2-10% of which Grave's disease is the commonest (Gebreyohannes et al. 2019). A retrospective study review of thyroid pathology of 222 (ratio of females to males, 7:1) thyroidectomies performed over three-year period between 1st January, 1999 and 31st December, 2001 at Kijabe Hospital in Kenya, revealed that prevalence of thyroid disorders are common in Kenya. It was noted in the review findings that malignancy rate was at 11.7% (papillary & follicular), multinodular goitre at 47% and Grave's disease rate at 13%. The findings also revealed that Grave's disease is common in rural Africa and malignancy is changing from the common papillary to follicular cancer, reflecting the widespread programme of iodinization of salt in Kenya (Hill et al. 2004). Efforts to eliminate Iodine deficiency disorder in Africa has been frustrated by civil conflicts, fragile political structures and poverty level (Assey et al. 2009). Despite salt iodination programme in Kenya, thyroid disorders, especially endemic goitre are still prevalent and remain an important Health problem (Kishosha et al. 2011). Nakuru level 5 Hospital is the largest Referral health facility at the central and south Rift valley regions in Kenya and cases both from within Nakuru County and neighbouring counties are handled at this facility. Despite this central role of this health facility, no studies have been conducted to characterize thyroid disorders. Research conducted elsewhere has shown that characterization of thyroid disorders can guide in the development of appropriate policy guidelines for the effective control, treatment modalities and management of these disorders. In Kenya however, such data on

characterization of thyroid disorders in this region is not readily available due to limited studies conducted so far, hence necessitated this study.

1.3 Justification

Thyroid disorders are common and more so among women in their middle ages causing morbidity in the productive fraction of the population. Among the thyroid disorders, which are common, data shows that goitre is prevalent in several areas of Kenya, especially highland areas of Rift valley region. The principal control strategy for iodine deficiency disorders in Kenya is the fortification of table salt. Studies have indicated that iodine prophylaxis is ineffective in reversing all types of goitre because not all goitres in Kenya are the direct result of iodine deficiency and there is a strong positive linear correlation between the presence of the goitrogens thiocyanate (CNS-) and existence of endemic goitre rates along KerioValley (Davies et al. 2008). Therefore, it is imperative that a study be conducted to characterize these thyroid disorders and to device control programme for early detection of those patients who will not respond satisfactorily to iodine supplementation. Some of the cases may not need iodine replacement, for example those that are due to the competitive and suppressive action of goitrogenic substances with iodine and the body's inability to utilize effectively the ingested iodide. Despite the central role played by Nakuru level 5 facility in handling thyroid cases both from within the surrounding communities and neighbouring counties, no studies have been conducted on thyroid disorders among patients at this facility. The present study seeks to characterize thyroid disorders among patients from this region and make data available to stakeholders. Availability of such data may lead to improved patient care and better prognosis. It will also facilitate and stimulate further studies on etiological factors.

1.4 Research Questions

1. What are the socio-demographic factors associated with the biochemical and non-biochemical characteristics of thyroid disorders among patients attending Nakuru level 5 Hospital surgical clinic?
2. What are the biochemical and non-biochemical characteristics of thyroid disorders among patients attending Nakuru level 5 Hospital surgical clinic?
3. What thyroid disorders are diagnosed at the Nakuru level 5 Hospital surgical clinic?

1.5 Objectives:

1.5.1 Broad Objectives

To identify socio-demographic factors associated with biochemical and non-biochemical characteristics of thyroid disorders and common clinical presentation among patients attending surgical clinic at Nakuru level 5 Hospital

1.5.2 Specific Objectives.

1. To determine the socio-demographic factors associated with biochemical and non-biochemical characteristics of thyroid disorders and common clinical presentation among patients at Nakuru level 5 Hospital.
2. To determine the biochemical and non-biochemical characteristics associated with common thyroid disorders among patients in the study area.
3. To determine the types and proportion of thyroid disorders diagnosed at Nakuru level 5 Hospital surgical clinic.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The thyroid gland, anatomy and physiology

The name thyroid is derived from Greek word *thyreoeides* meaning shield shape. The thyroid gland is a butterfly shaped and brownish-red gland in the front of the neck, positioned just below the larynx and in front of the trachea and are part of glands of Endocrine system (Guyton et al. 2011). It weighs 25g to 30g but it is larger in women. Its size increases during menstruation and pregnancy. The right and the left lobes connected by a narrow isthmus that extends interiorly across the front of the neck (Gray et al. 2008). In about 80% of individuals, the pyramidal lobe exists ascending from the isthmus to the hyoid bone. Histologically, the thyroid is composed of closely packed sacs called follicles which consist of follicular cells and colloid. Follicular cells secrete the thyroid hormones thyroxine and tri-iodothyronine, T_4 and T_3 respectively (Gartner et al.2010). The colloid is a jellylike substance inside the follicles and contains iodinated thyroglobulin. There are also parafollicular cells that secrete calcitonin located in the upper poles of the thyroid lobes reflecting their origin as neuroectodermal cells derived from the ultimobranchial bodies and are part of the amine containing precursor decarboxylase (Gartner et al. 2010). The physiology of the thyroid gland involves a cascade of metabolic activities controlled by hormones which includes, breathing, heart rate, central and peripheral nervous systems, body weight, muscle strength, menstrual cycles, body temperature, cholesterol levels etc. Iodine is the raw material for thyroid hormones synthesis (Melmed et al. 2011). The minimum intake of iodine per day for normal thyroid function is 150ug of which 120ug enter thyroid at normal rates of hormone synthesis and secretion, therefore reduce intake of iodine is responsible for the widespread endemic goitre

(Organization et al 2014). Sources of iodine include but not limited to seafood, bread, dairy products, iodized salts and also iodine supplements. Upon ingestion, iodine is first converted to iodide for absorption. The thyroglobulin is a glycoprotein synthesized in the thyroid follicles and secreted into the colloid by exocytosis. It is bound to thyroid hormone till it is secreted into the blood after which it ingested back into the colloid (Schomburg et al. 2008). Thyroid Peroxidase (TPO) enzyme catalyzes oxidation of iodide and its absorption. Therefore, any immunoglobulin that acts against thyroid peroxidase enzymes and thyroglobulin protein will be evident of Hashimoto's disease. The active form of thyroid hormone, T_3 is required for the functioning of almost all the tissues in the human body. In healthy people, the gland chiefly secretes tetra-iodothyronine (T_4) which is transformed into active tri-iodothyronine (T_3) in other organs by the selenium-dependent enzymes iodothyronine deiodinase, D1 and D2 (Maia et al. 2011). Tri-iodothyronine attaches to the thyroid hormone receptor within the cell nucleus and activates the turning and expression of selected genes and the production of specific gene products (proteins). Furthermore, the hormone, T_3 attaches to Alpha-v beta-3 on the cell membrane and stimulating the sodium / hydrogen anti-port and process of blood vessels formation and cell proliferation.(Cheng et al. 2010). Nearly 99.7% of thyroid hormone in blood circulation is bound to thyroxin-binding globulin (TBG); only the free unbound thyroid hormone is biologically active (Garber, Cobin et al. 2012). Overexpression of type 3 deiodinase (D3) can thus lead to consumptive hypothyroidism (Pasa et al. 2017).The human body derives its thyroid hormones from the thyroid gland as the only source. Formation of thyroid hormones in the body requires the presence of iodine and tyrosine as an amino acids. The element iodine in the blood circulation is absorbed by the thyroid gland and integrated into thyroglobulin protein and this

process is controlled by the thyrothropin (TSH), which is secreted by the pituitary. Not enough iodine, or TSH, can result in decreased production of thyroid hormones (Gaitonde et al. 2012). The maintenance of thyroid hormone levels within normal values is by hypothalamic- pituitary- thyroid (HPT) axis and the production of thyrothropin by the anterior pituitary gland is triggered in turn by TRH, produce from the hypothalamus. Thyroxine, by negative feedback mechanism control the release and production of TSH and TRH. Decrease production of TRH, which is not common can lead to reduce TSH production, hence decrease thyroid hormone production (Koulouri et al. 2013). Pregnancy results to noticeable and apparent physiological changes in thyroid hormone. Iodine requirements are increased and the thyroid gland is increased in size by 10% while thyroxine hormone production is elevated by 50%. Many women develop hypothyroidism before or after giving birth or are iodine deficient but with normal thyroid function, have also evidence of immunological auto-immunity. (Stagnaro-Green et al. 2011). Synthesis of thyroid hormone include: iodine trapping, oxidation, iodination, coupling, storage and lastly, release. The first three steps are catalyzed by TPO (Gray et al. 2008).

2.1.1 Iodine role in thyroid gland physiology

The micro-element / micro-nutrient iodine found in soil and water is consumed in various chemical forms. Iodine is reduced to iodide in the stomach. The reduced iodine is almost completely assimilated in the gut and duodenum (Zimmermann et al. 2009). Iodide is removed from the circulatory system first and foremost by the thyroid and kidney. Plasma iodide has a half-life of roughly ten hours under normal conditions but is reduced if the thyroid is hyperactive, as in iodine deficiency disorders or hyperthyroid conditions. In iodine-sufficient areas, the mean daily turnover of iodine by the thyroid gland is roughly 80-95 µg in adults under normal

circumstances. In healthy adult persons their bodies contain 15 to 20 mgs of iodine of which 70% - 80% is concentrated in the thyroid gland. In the basolateral cell membrane which is the fraction of the plasma membrane of the thyroid gland cells contain sodium / iodine symporter (NIS) which transfers iodide into the thyroid follicular cells across a concentration gradient 20-50 folds that of plasma by active transport (Zimmermann et al. 2009). Breakdown of thyroxine and tri-iodothyronine hormones in the periphery tissues releases iodine that re-enters the plasma iodine pool. Majority of the absorbed iodine is finally excreted in the urine and only a small quantity is passed in the faeces.

2.1.2 Control of the thyroid gland by iodine

Thyroid function status as been known to be controlled by iodide concentration in the body. The effect of iodide is to reduce the response of the thyroid gland to thyroid stimulating hormone (TSH); to markedly hinder its own oxidation, to reduce its trapping effect after a delay; and, at elevated concentrations, to hinder thyroid hormone secretion and release. A small change in iodide intake is enough to adjust the thyroid system at different serum thyrotropin levels. The modulation of the thyroid response to thyrotropin by iodide plays a significant role in the negative feedback mechanism loop (De Groot et al. 2013). Iodine organification increases at first and then decreases in response to increasing doses of iodide ingestion. The, termed 'Wolff-Chaikoff effect' (acute inhibition of organification) follows the administration of high concentration of inorganic iodide within the thyroid cells. The mechanism and the process responsible for Wolff-Chaikoff effect' is not well understood but it may be due to inhibitory effect of iodide on thyroid peroxidase enzyme or other enzymes in the thyroid gland. In normal individuals who have ingested iodide, the inhibition process of organification is short live and this occurrence is termed 'escape from the

Wolff-Chaikoff effect' or adjustment to the Wolff-Chaikoff effect. Iodide has been known to have an inhibitory effect in various metabolic processes in the thyroid cells. Iodide also hinders the cyclic adenosine monophosphate (cAMP) cascade and the Ca^{2+}-phosphatidylinositol 4, 5-bisphosphate (PIP_2) biochemical cascade. Iodide also stimulate hydrogen peroxide production and protein iodination in the thyroid cells of some species, including human-beings (De Groot et al. 2013). The down-regulation process of sodium-iodide symporter (NIS) by iodide describes the adaptation to the Wolff-Chaikoff effect.

2.1.3 Effect of excessive iodine on the thyroid gland

Excessive iodide ingestion can affect thyroid function status. Most people can tolerate high dietary intakes of iodine conspicuously well. The synthesis of thyroid hormones, T_4 and T_3 are inhibited by the acute Wolff-Chaikoff effect following subjection to high iodide doses. Thyrotoxicosis can manifest itself following administration of supplemental iodide to subjects with endemic iodine deficiency goitre. The termed iodide-induced hyperthyroidism response, or the Jod-Basedow effect (Jod derived from the German word for "iodine"), occurs in a small fraction of individuals who are prone and at risk. Patients with underlying medical conditions especially, mild, autoimmune thyroid disease, like Hashimoto's thyroiditis, are particularly vulnerable to developing iodine-induced hypothyroidism during period of exposure (Kovacs et al. 2011)._The Wolff-Chaikoff effect dose does not develop until 36-40 weeks gestation, hence, preterm infants are susceptible to the effects of iodine overload (Chung et al. 2009). Increase iodide intake has a direct link and association with autoimmune thyroid disease (Laurberg et al. 2010). A sharp increase in iodide intake in an iodine-deficient individuals may induce thyroid autoimmunity. Population of people with anti-thyroid antibodies have a higher chances of risk factors developing

thyroid dysfunction when the iodide intake is increased (Li et al. 2008). Iodide consumption does not appear to influence the overall incidence of thyroid cancers in the populations. Increase consumption of iodide in high iodine areas is associated with thyroid dysfunction in children (Sang et al. 2013).

2.1.4 Physiological significance of the plasma inorganic iodide for normal thyroid Function

The plasma inorganic iodine has been studied and estimated prospectively by an Isotope dilution technique in males and females without evidence of thyroid disease. Significant findings were observed as follows: The mean plasma inorganic iodine is the same in both sexes at 18.0 µg per 100mls of plasma (Zimmermann et al. 2009). Females show significant negative correlation between plasma inorganic iodine and thyroid radio-iodine clearance. There is positive correlation between plasma inorganic iodine and absolute iodine uptake (AIU) in both sexes suggesting that the higher the plasma inorganic iodine, the more iodide is trapped by thyroid gland per unit time. There is significance positive correlation of plasma inorganic iodine with age in females but not males. Females but not males show a negative correlation between plasma inorganic iodine and renal iodide clearance. The study concluded that there are differences in iodine metabolism between males and females and this might contribute to the greater incidence of simple goitre in females. Another study showed that the administration of large amount of iodine temporarily inhibit the capacity of the normal thyroid gland to bind iodine organically (De la Vieja et al. 2000).This inhibition is related to the level of plasma iodine. So long as the concentration of plasma iodine exceeds 20 – 35% of the plasma normal values, no organic binding of iodine occur in the gland, and only when the concentration fell below this critical range, did the gland resume its functions of depositing iodine in an organic form.

From these findings, it was postulated that the level of plasma inorganic iodine is part of a homeostatic mechanism governing hormone synthesis in the normal gland. Thus when a large amount of iodine is ingested, the prevention of its deposition as organically bound compound in the thyroid gland keeps the ingested iodine circulating as an inorganic iodide form, which is readily excreted by the kidneys.

2.2 Thyroid hormones synthesis

Thyroid hormones (THs) play critical roles in differentiation, growth, metabolism and physiological function of virtually all tissues. Thyroid hormone is required for the normal function of nearly all tissues, with major effects on oxygen consumption and metabolic rate (van der Spek et al. 2017). Disorders of the thyroid gland are among the most common Endocrine ailments. Endemic cretinism caused by iodine deficiency is still a public health issue in poor and developing countries (Zimmermann et al. 2011; van der Spek et al. 2017). Thyroid hormones synthesis and secretion are controlled by a negative-feedback mechanism that entails the hypothalamus, pituitary, and thyroid gland [hypothalamic/pituitary/thyroid (HPT) axis] (Chiamolera et al. 2009). Thyrothropin releasing hormone (TRH) is a tripeptide (PyroGlu-His-Pro) synthesized in the paraventricular nucleus of the hypothalamus. It is conveyed through axons to the anterior pituitary. TRH attaches to TRH receptors in pituitary thyrotropes, a subpopulation of pituitary cells that secrete thyrotropin hormone. Thyrotropin hormone is a 28-kDa glycoprotein composed of α- and β-subunits designated as glycoprotein hormone α- and TSH β subunits. Thyrothropin (TSH) is the key regulator of thyroid hormones release and secretion. It plays an important role in thyroid growth and development. TSH binds to the TSH receptor (TSHr), which also is a seven-transmembrane spanning receptor coupled to G_1 protein (Kochman et al. 2014). Stimulation of thyrotropin receptor by TSH or autoantibodies

in Graves' disease results in an increase in intracellular cAMP and activation of protein kinase A-mediated pathways. Both Thyrothropin releasing hormone and thyroid stimulating hormone secretion are negatively controlled by thyroid hormones, T_4 and T_3 in addition to somatostatin and dopamine from the hypothalamus. Thyroid hormones, T_4 and the more potent T_3, are synthesized in the thyroid gland. A number of thyroid genes, including Na^+ / I^- symporter (NIS), co-transporter of $2Na^+$ & I^-, thyroglobulin (Tg) protein, and thyroid peroxidase (TPO) enzyme, are activated by TSH and promote the synthesis of thyroid hormones. Iodide and sodium ions are actively transported and concentrated into the thyroid gland by NIS against an electrochemical gradient. It has been shown that goitrogens such as thiocyanate and per chlorates block this stage (Gray et al. 2008).

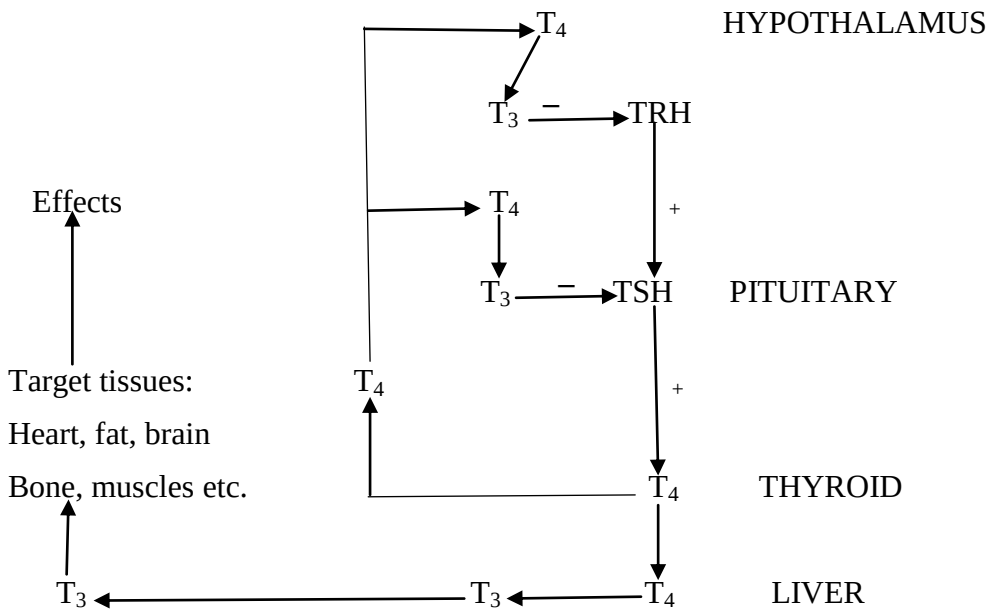


Figure 1 : Regulation of thyroid hormones within Hypothalamus-Pituitary-Thyroid axis (HPT) by positive and negative mechanisms (Ortiga-Carvalho et al., 2016)

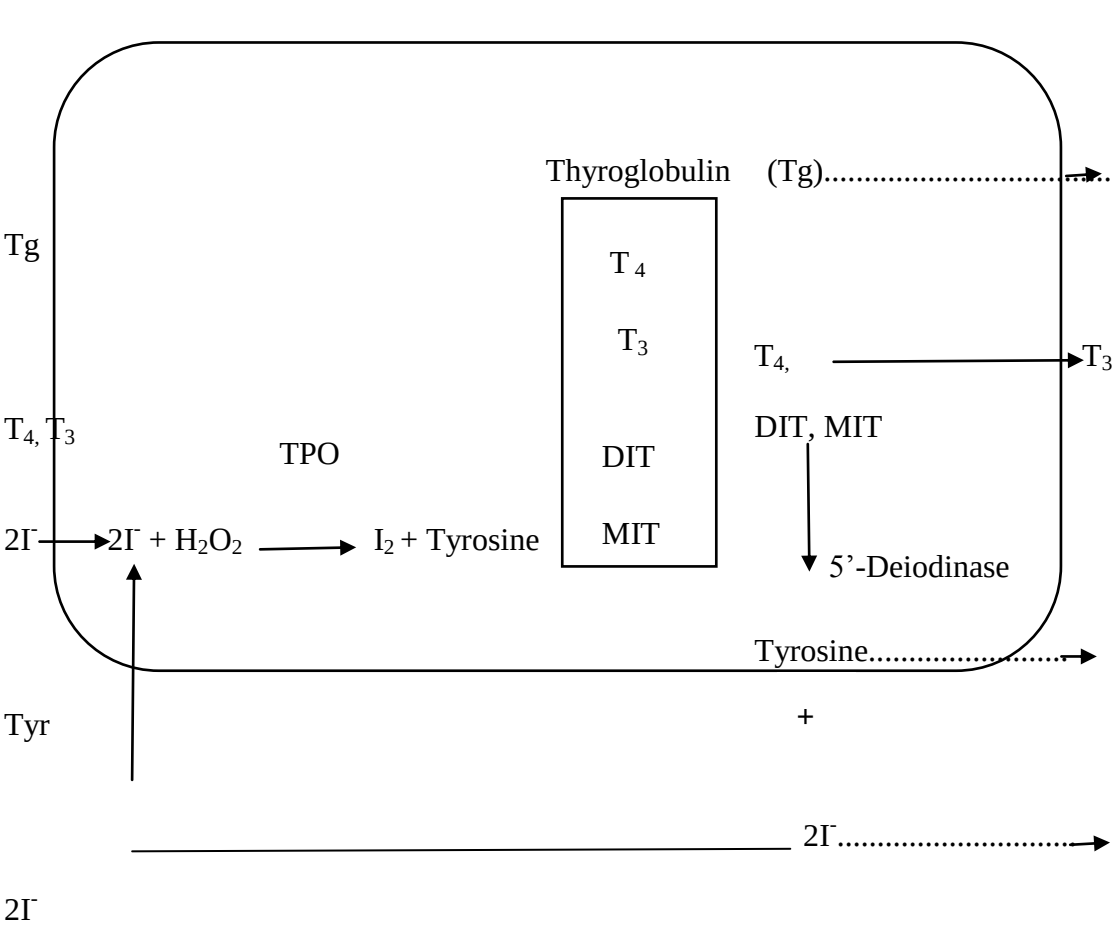


Figure 2: Thyroid hormone synthesis in thyroid follicle (Carvalho & Dupuy, 2017)

In oxidation, the trapped iodide is oxidized by Thyroid peroxidase (TPO) enzyme in the presence of hydrogen peroxide (H_20_2) to form iodine, which combines with the amino acid tyrosine residue and incorporated to a 660 Kilo Daltons (molecular weight)-glycoprotein, thyroglobulin (Tg). This iodination of specific tyrosine located on thyroglobulin yields 3-monoiodinated and 3, 5-diiodinated residues (MIT, 3-monoiodo-tyrosines; DIT, 3, 5-diiodo-tyrosines) that are enzymatically coupled to form 3, 5, 3, 5-tetra-iodothyronine T_4 and 3, 5, 3-tri-iodotyrosine, T_3. The iodinated thyroglobulin containing monoiodo-tyrosines diiodo-tyrosines, thyroxine and tri-iodothyronine, then is stored as an extracellular storage polypeptide in the colloid within the lumen of thyroid follicular cells. Genetic abnormalities along the synthetic pathway of thyroid hormones have been recounted and expressed in humans and are major underlying causes of congenital hypothyroidism in iodine sufficient environments (Ghasemi et al. 2013).

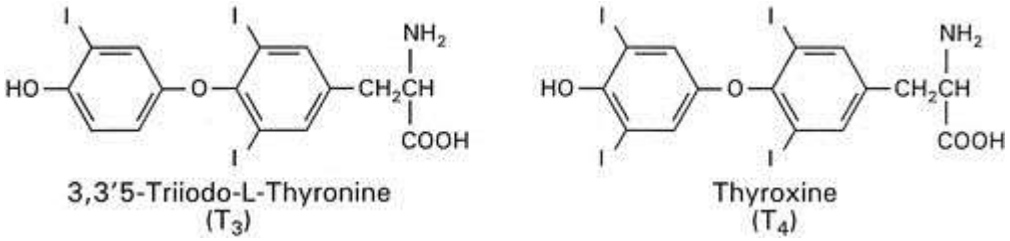


Figure 3: Structural difference between the thyroid hormones T_3 and T_4 (Retrieved January 10, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Thyroid-hormones)**.**

Thyroid hormones are derivatives of the amino acid tyrosine bound covalently to iodine. The two principal thyroid hormones are: Thyroxine (T_4) or L-3, 5, 3', 5'-tetraiodothyronine and (T_3) or 3, 3'5-triiodo-L-thyronine. Thyroid hormones are basically two tyrosine linked together with the critical addition of iodine at three or four positions on the aromatic rings. The number and position of the iodine is

important. Several other iodinated molecules are generated that have little or no biological activity; so called "reverse T_3" (3, 3', 5'-T_3). A large majority of the thyroid hormone secreted from the thyroid gland is T_4, but T_3 is the considerably more active hormone. Although some T_3 is also secreted, the bulk of the T_3 is derived by deiodination of T_4 in peripheral tissues, especially liver and kidney. The secretion of thyroid hormones requires endocytosis of the stored iodinated thyroglobulin from the apical surface of the thyroid follicular cell. The internalized thyroglobulin is incorporated in phagolysosomes and undergoes the process of proteolytic digestion, recover of MIT and DIT, and release of T_4 and T_3 into the circulation via the basal layer. A greater number of released thyroid hormones is in the form of T_4, as total serum T_4 is 40 times greater in concentration than serum T_3 (90 vs. 2 nM). About 0.03% of the total serum T_4 is free (unbound), with the remainder (99.97%) bound to carrier proteins e.g. albumin, thyroid binding prealbumin and thyroxine binding globulin (TBG), a glycoprotein synthesized in the liver. Roughly 0.3% of the total serum T_3 is free, with the remainder bound to the principle carrier thyroxine binding protein (TBG) and albumin. It is the free active (T_3) thyroid hormone that enters target cells and generates a physiological response. The principal pathway for the production of T_3 is through 5′-deiodination of the outer ring of T_4 by deiodinase (D1, D2) and accounts for the majority of the circulating T_3 (Mondal et al. 2016). Type I deiodinase (D1) is located in peripheral tissues e.g. liver and kidney and accounts for the conversion of most T_4 to T_3 in circulation. Type II deiodinase (D2) is found in brain, pituitary, and brown adipose tissue and primarily converts T_4 to T_3 for intracellular use. Although thyroid hormones especially the more potent T_3, exert their effects on a number of intracellular loci, their main effect is on the transcriptional regulation (increase / decrease expressions) of target genes. Studies showed that the

effects of thyroid hormones at the genomic level are mediated by nuclear thyroid receptors, which bind thyroid hormones with high affinity and specificity (Cheng et al. 2010). Analogous and related to steroid hormones that are also nuclear receptors, thyroid hormones enter the cell through membrane transporter proteins and proceed to the nucleus. It then binds to thyroid receptors, which may already be prebound to Thyroid hormone response elements (THREs) or transcription factor-binding site, located in promoter regions of target genes. The formation of ligand-bound Thyroid receptor (TR) complexes that are also bound to THREs in the promoter region of the DNA, is the crucial and initial step in the positive (up-regulation) or negative (down-regulation) of target genes and the consecutively regulation of protein synthesis (gene products). Thyroid receptors can be regarded as ligand-regulatable transcription factors given their abilities to bind both ligand (T_3) and DNA as well as regulate gene transcription,

2.3 Molecular Mechanism of thyroid hormone action

The active form of the thyroid hormone tri-iodothyronine $T_{3,}$ after its activation from the prohormone thyroxine $T_{4,}$ regulates a wide range of genes in humans (Gereben et al. 2008). Regulation of the signaling pathways is highly complex due to the expression of multiple thyroid hormone receptor (TR) isoforms, cell and tissue-specific thyroid hormone transporters and interactions with co-repressors and co-activators (Cheng et al. 2010). In most cases, thyroid signals are involved in cross-communication and interaction with a range of other signaling pathways (Liu et al. 2010). The anterior pituitary gland in response to feedback mechanism from the circulating thyroid hormone T_4, secretes thyroid stimulating hormone (TSH) which acts directly on the TSH receptor (TSH-R) expressed on the thyroid follicular cell basolateral membrane (Chiamolera et al. 2009). TSH, also called thyrotropin hormone

controls the uptake of iodide mediated by the sodium / iodide symporter, followed by a series of steps required for normal thyroid hormone synthesis and release (Kopp et al. 2012).

Thyroid hormones (THs) play critical roles in physiological functions of virtually all mammalians' tissues (Tata et al..2013) and is required for amphibian metamorphosis (Furlow et al. 2006), tremendous progress has been made in understanding the molecular mechanisms that underlie thyroid hormone action. Thyroid Receptor (TR) genes has two major isoforms, TRα and TRβ, with different patterns of expression in development and in adult tissues (Cheng et al. 2010).

Thyroid hormone, tri-iodothyronine (T_3) regulates gene expression by binding to high affinity nuclear receptors that are ligand-regulatable transcription factors belonging to the nuclear hormone receptor superfamily. Recognition of the specific thyroid hormone response element (THRE) sequences in the promoter region of T_3-target genes by thyroid hormone receptors (TRs), leads to its activation or repression of gene transcription in response to hormone. Thus the study of thyroid hormone (T_3) action has important biological and medical implications.

Tri-iodothyronine (T_3), the active form of prohormone thyroxine is produced by de-iodination of thyroxine (T_4) by the enzymes 5'-deiodinase (5'-D), types I and II (Cheng et al. 2010). Type I (D1) deiodinase is found in peripheral tissues such as liver and kidney and is responsible for the conversion of the majority of T_4 to T_3 in circulation and two thirds of the total T_3 in the body. Type II (D2) deiodinase is found in brain, pituitary, and brown adipose tissue and primarily converts T_4 to T_3 for intracellular use. T_3 enters the cell or is produced locally and then transported into the nucleus. The active T_3 hormone binding to the ligand-binding domain in the thyroid receptor results in the conformational change and movement of the carboxyterminal

helix 12, displacement of co-repressor binding, and promotion of co-activator binding, which then leads to recruitment of RNA polymerase II and initiation of gene transcription. Transcriptionally active forms of thyroid hormone receptors (TR) include monomers, homodimers, and heterodimers with nuclear protein partners, such as the retinoid X receptor (RXR). The T_3-receptor complex interacts with specific sequences in DNA called Thyroid hormone response elements (THRE) regulatory regions and modifies gene expression. T_3 causes both increases and decreases in gene expression and may also influence the stability of messenger RNA (mRNA). 9-cis RA denotes 9-cis-retinoic acid, the ligand for RXR (Schroeder et al. 2014). The clinical manifestations of thyroid hormone excess and deficiency are examples of the myriad actions of the hormone. Thyroxine (T_4), the primary secretory product of the thyroid, is relatively inactive and is converted to the active hormone, tri-iodothyronine (T_3).

Figure 4 below summarizes the Mechanism of thyroid hormone action in the cell.

I. Receptors for thyroid hormones are intracellular DNA-binding proteins that function as hormone-responsive transcription factors, very similar conceptually to the receptors for steroid hormones.

II. Thyroid hormone T_3, enter cells through membrane transporter proteins

III. T_3 binds to ligand-binding domain of Thyroid Receptor (TR)

IV. The hormone- receptor complex then binds to Hormones Response Element (HRE) of the DNA via zinc fingers in the promoter region of the target DNA and increases / decreases gene expressions - synthesis of functional gene products (proteins)

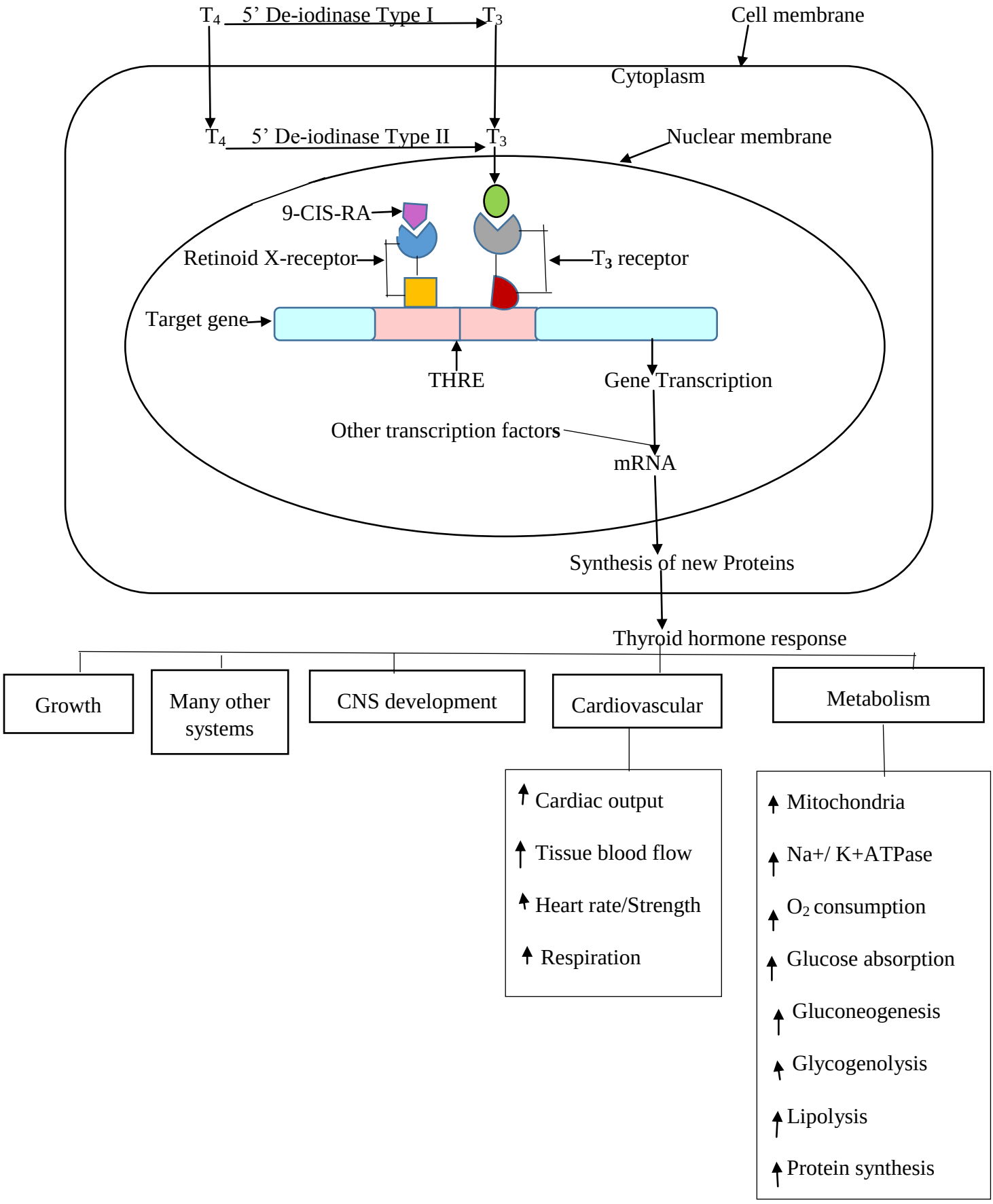


Figure 4: Action of Thyroid Hormone in Cell nuclei (Passmore et al. 1961) modified

2.3.1 Thyroid Hormone Receptor (T_3)

Thyroid hormones receptors (T_3Rs) are intracellular nuclear DNA-binding proteins that function as hormone-responsive transcription factors ($HRTF_s$), similar in functions and related to the receptors for steroid hormones. Thyroid hormones enter cells either by diffusion or by carrier-mediated transporter proteins. T_3 receptors are members of a family of hormone-responsive nuclear transcription factors (HRNT) that are similar in structure and mechanism of action (Cheng et al. 2010). The functional domains of the receptor include a carboxy-terminal portion, which is important for ligand binding and interactions between receptors, a DNA-binding domain, and an amino-terminal domain, which does not have an identified functional role. The domain that interacts with DNA includes two sequences of amino acids with cysteine residues that form loop structures and chelate a zinc atom, termed "zinc fingers" amino acids within this structure serve to determine the specific DNA sequence to which several monomeric receptor binds; others are important for receptor dimerization, the usual functional form for positive regulation. The nature of the DNA sequences that bind T_3 receptors is central to determining which genes are stimulated or inhibited by T_3. In vitro, T_3 receptors bind to DNA as homodimers or as heterodimers with other nuclear proteins, such as the retinoid X receptor (whose ligand is 9-cis-retinoic acid). T_3 disrupts the binding of T_3-receptor homodimers to DNA, but not that of T_3-receptor heterodimer. The ligand-binding domain confers specificity for T_3, and mutations in this domain can result in tissue resistance to thyroid hormone (Pawlak et al. 2012). The binding of a steroid hormone e.g. thyroid hormone to its receptor causes a conformational change in its receptor that uncovers its zinc finger, DNA – binding domain. There are two T_3-receptor genes, α and β, located on chromosomes 17 and 3, respectively. The α1 and β T_3 receptors bind T_3

and can activate a T_3-response element in vitro, but they differ in potency and affinity for T_3 analogues. There are at least two alternative messenger RNA (mRNA) splice products for each gene. The major products of the T_3-receptor-α gene include T_3 gene, T_3 receptor β1 and β2 α1 and α2, the latter does not bind T_3 (Vennstr ÖM et al. 2010). This variant is expressed in most tissues, especially in the brain, and may inhibit binding of T_3 receptors to DNA. The products of the T_3-receptor-β, binding of protein to DNA is through structural motifs e.g. zinc finger, leucine zippers and helix turn helix.

2.3.2 Phosphorylation of thyroid hormone receptors (T_3R)

Recent studies have observed that increasing the phosphorylation state of cells can enhance T_3-mediated transcriptional activation of target genes (Moeller et al. 2011). The phosphorylation in transcriptional activation has been demonstrated that T_3R can be phosphorylated in vitro and in vivo. Chick T_3Rα-1 has at least two serine phosphorylation sites in the amino-terminal A/B domain, but the functional role(s) is not known. Additionally, these sites do not appear to be conserved across species. The human T_3Rß-1 can be phosphorylated in vivo and in vitro mechanisms for this enhanced transcriptional activation are not known but may involve phosphorylation of T_3R, RXR, or co-activators (Oltová et al. 2010). In support of the potential role of T_3Rp, the phosphorylation sites have not been determined. Studies have used HeLa cytosol extract for in vitro to phosphorylate Escherichia coli-expressed T_3Rβ-1 and examined the binding of phosphorylated T_3Rβ-1 to several THREs and found that phosphorylation selectively enhanced T_3R homodimer, but not T_3R / RXR heterodimer, binding to several different THREs. Phosphorylation enhanced DNA binding by both T_3R complexes. Phosphorylation by protein kinase A can decrease chick T_3R α-1 monomer binding to THREs. These results suggest that

phosphorylation may be another mechanism, in addition to T_3 binding, that can modulate T_3R complex binding to THREs. Additionally, T_3 itself can modulate the phosphorylation state of T_3R (Oltová et al. 2010)

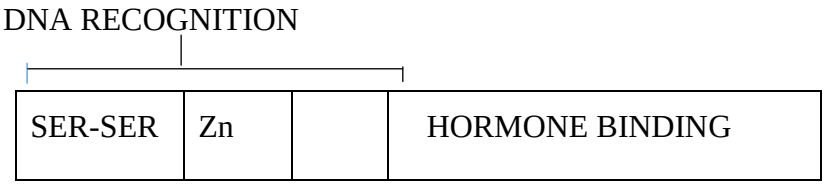


Figure 5: Region of T_3R α-1 (Receptor) (Tzagarakis-Foster & Privalsky, 1998).(Carvalho & Dupuy, 2017)

2.4 Thyroid Disorders

Thyroid disorders are conditions that affect the thyroid gland. The role of the thyroid gland is to regulate metabolic process throughout the body. Different types of thyroid disorders affect either the 'structure' (shape) or 'function' and are basically characterized by either abnormal thyroid function such as Hypothyroidism and hyperthyroidism or by thyroid enlargement such as goitre and thyroid nodules which manifest as a structural thyroid disorders (Melmed et al. 2015). Thyroid gland utilizes the trace element iodine to synthesis thyroid hormones, T_4 & T_3. Function of thyroid gland is regulated by feedback mechanism involving the brain. When thyroid hormones levels are low, the hypothalamus in the brain produces thyrothropin releasing hormones (TRH) which cause pituitary gland to release thyroid stimulating hormone TSH, which stimulate the thyroid gland to release more T_4. Disorders of pituitary gland and hypothalamus affect thyroid functions and causes thyroid disorders.

2.5 Characteristics of thyroid disorders

Thyroid disorders are characterized by two factors: changes in the "function" of the thyroid gland and changes in the "shape" / ‘Structure’ of the thyroid gland. Some disorders are marked by both factors, while others are marked by only one of the two factors (Ogbera et al. 2011). Several different disorders can arise when the thyroid gland produces too much circulating hormones (hyperthyroidism) or insufficient hormones (hypothyroidism). The four commonly encountered disorders of the thyroid are Hashimoto’s disease (thyroiditis), Graves’s disease, thyroid nodules and goitres.

2.6 Pathophysiology of Common thyroid disorders

2.6.1 Hypothyroidism (Underactive thyroid gland)

Also known as chronic lymphocytic thyroiditis. It is thought to be due to combination of genetic and environmental factors. Hypothyroidism is due to insufficient functioning of the thyroid gland itself and does not produce enough thyroid hormones to meet the needs of the body *(*Primary thyroid gland failure / primary hypothyroidism), insufficient stimulation to evoke a response by thyroid-stimulating hormone from the pituitary gland (Pituitary hypothyroidism / secondary hypothyroidism), or insufficient release of thyrothropin-releasing hormone from the brain's hypothalamus (tertiary hypothyroidism). Primary hypothyroidism is about a thousand fold more common than central hypothyroidism. Women, especially those older than age 60 years, are probably prone to suffer from hypothyroidism. Hypothyroidism disturbs the usual and standard balance of chemical reactions in the body. Prolong and untreated hypothyroidism can contribute to a number of health related problems, e.g. infertility, obesity, joint pain, and coronary heart disease. Iodine deficiency is the leading cause of primary hypothyroidism and accounts for many cases of endemic goitres worldwide. In many areas of the world with adequate dietary

iodine intake, hypothyroidism is typically caused by Hashimoto's thyroiditis (chronic lymphocytic thyroiditis) of the autoimmune thyroid diseases. Hashimoto's may be associated with a goitre. Hashimoto's thyroiditis is marked by infiltration of the thyroid gland cells (thyrocytes) with T-lymphocytes and auto-reactive antibodies directed against particular thyroid antigens namely: thyroid peroxidase enzyme, thyroglobulin protein and the thyrotropin receptor. Hypothyroidism affects 3-10% of adults, with a higher incidence in women and the Elderly (Vanderpump et al. 2011). Among the factors that contribute to hypothyroidism are listed below and include:

Treatment for hyperthyroidism: Individuals whose thyroid gland produces too much thyroid hormone (hyperthyroidism) are usually treated with radioactive iodine or anti-thyroid drugs to conform and manage thyroid function status of the gland. Although, in some circumstances, treatment of hyperthyroidism can lead to permanent state of hypothyroidism.

Thyroid surgery: Excision of all or a large portion of thyroid gland can results in the decline or cessation of hormone production and in this case patients are required to take thyroid hormone replacement for life.

Radiation therapy: Radiation treatment directed at head and neck cancers can affect the functioning of thyroid gland and can cause hypothyroidism to the concern patients.

Medications. Medications such as lithium, used to treat psychiatric diseases contribute to hypothyroidism

Less common causes are:

Congenital disease. Children born with faulty thyroid gland or no thyroid gland at all due to genetic defects inherited in their development stages, have and suffer from congenital hypothyroidism. Such children appear normal at birth and thus why most countries now require newborn thyroid screening.

Pituitary disorder (Secondary hypothyroidism): Pituitary disorder, although a rare condition cause of hypothyroidism is due to inadequacy of the pituitary gland to produce enough thyroid-stimulating hormone (TSH), normally due to benign tumor of the pituitary gland.

Pregnancy: Postpartum hypothyroidism develops in some women during or after pregnancy often because they produce antibodies to their own thyroid gland. If left unmanaged, hypothyroidism escalates the dangers in developing premature delivery, miscarriage and pre-eclampsia (condition that causes a notable rise in a woman's blood pressure during the third trimester) which can also seriously affect the growth and development of fetus,

Iodine deficiency: The trace element (micronutrient) iodine, which is essential for the production of thyroid hormones is primarily found in seafood, seaweed and other plants grown in iodine-rich soil. Iodine can also be found in household iodized salt. In several countries of the world, iodine deficiency is prevalent but the household consumption of iodized table salt has effectively eliminated this problem. On the other hand, ingesting in too much iodine can cause hypothyroidism **(**'Wolff-Chaikoff effect'- iodine induced organification inhibition).

Autoimmune disease: Common causes of primary hypothyroidism are atrophic auto-immune thyroiditis and Hashimoto's disease which are characterized by the body's

own immune system attacking the thyroid gland leading to inflammation (chronic lymphocytic thyroiditis) causing it to decay, resulting to underactive thyroid gland resulting in hypothyroidism (Melmed et al. 2015).

People who develop Hashimoto's thyroiditis have the most common cause of hypothyroidism. Autoimmune disorders occur when body's immune system produces antibodies that attack its own tissues. Sometimes this process involves the thyroid gland. Researchers and experts cannot comprehend why the body can produce antibodies against self. Various theories and postulations have been put forward by different teams of scientists. Some thought a virus or bacterium might activate the response, while others suspect a genetic defect may be involved. Nearly all autoimmune diseases originated from more than one factor. Even so it happens, these auto-antibodies upset the thyroid's ability to produce hormones. Hashimoto's thyroiditis is an organ-specific, auto-immune T-cell mediated disease affecting thyroid follicular cells (thyrocytes) and characterize with presence of anti-TPO and anti-Tg antibodies with low level of circulating T_4 and T_3. Maturation of the auto-reactive T & B lymphocytes and mediated pathway through IL-1, IL-6 leading to production of auto-reactive CD4 T-cells & CD8 cytotoxic T-cells and Ig G auto-antibodies. Th1 mediated mechanism produces IL-12, TNFα, IFγ and FasL from cytotoxic T-cells plays a major role in the activation of cytotoxic-T lymphocytes, CD8+ T cells in response to cell-mediated immune response effected by helper T-lymphocyte CD4+ T cells which is central to thyrocytes destruction and apoptosis respectively.. Histologically, there is accumulation of lymphocytes and plasma cells. Hashimoto's thyroiditis is also associated with type I diabetes and rheumatoid arthritis (Ajjan et al. 2015).

Secondary hypothyroidism is due to anterior pituitary deficiency and tertiary hypothyroidism is due to hypothalamic deficiency. The essential difference in laboratory findings between the primary, secondary and tertiary forms is the level of plasma TSH. This is high in primary and low in secondary and tertiary hypothyroidism (Yamada et al. 2008). Clinical manifestation of hypothyroidism include tiredness, forgetfulness, depression, lack of concentration, weight gain, cold intolerance, elevated cholesterol, puffy eyes, enlarged thyroid, hoarseness of the voice, sore throat, irregular and heavy menstruation, dysphasia, constipation, muscle weakness, bradycardia and skin dryness (Persani et al. 2012).

Diagnosis:

The first step for screening any type of thyroid disorders is measuring the level of thyroid stimulating hormone (TSH). TSH is raised in primary hypothyroidism and low in secondary hypothyroidism. Measuring levels of circulating thyroid hormones T_4 and T_3, which in this case is low. Determine the presence of thyroid auto-antibodies levels, anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Hg) (Gharib et al. 2010).

Treatment: Hormone-replacing medication (Levothyroxine) to raise the levels of T_4 & T_3 or lower TSH (Ross et al. 2016)

Subclinical hypothyroidism

This is a milder form of hypothyroidism marked by raised serum TSH levels, and with a normal serum thyroxine, T_4 levels (Bona et al. 2013). Hashimoto's thyroiditis is the leading cause of this milder form of hypothyroidism (Baumgartner et al. 2014).

Diagnosis of this milder form of hypothyroidism in adults is detected when TSH levels are greater than 5 µIU/L and less than 10 µIU/L (Dons et al. 2009). The clinical

presentation of subclinical hypothyroidism is varying and definitive features of hypothyroidism may not be noticed (Bona et al. 2013). Of persons with subclinical hypothyroidism, a good percentage normally develop overt hypothyroidism (low T_4 and T_3) each year.

Individuals in the population with detectable antibodies against thyroid peroxidase (TPO), are present in 4.3%, while in those individuals with no detectable antibodies, against thyroid peroxidase, occurs in 2.6% (Garber et al. 2012). People diagnosed with subclinical hypothyroidism and detectable anti-TPO antibodies and who do not require treatment should be subjected to repeated thyroid function tests every year in contrast to those who do not exhibit antibodies (Forehan et al. 2012). During gestation period, the thyroid gland increases the production of thyroid hormone by 50% more to provide enough hormone for the growing fetus and the expectant mother (Negro et al. 2014). In pregnant mothers, free thyroxine levels may be decreased than expected due to increased binding to thyroid binding globulin (TBG) and decreased binding to albumin. These values should either be corrected for the stage of gestation (Stagnaro-Green et al. 2011), or total T_4 levels should be used instead for detection and diagnosis purposes (Garber et al. 2012). In pregnant women, subclinical hypothyroidism is defined as a TSH levels range between 2.5 and 10 µIU/L with thyroxine levels within their normal ranges while those with TSH above 10 µIU/L are deemed to be overtly hypothyroid even if the thyroxine levels are within their normal ranges (Stagnaro-Green et al. 2011). Auto-antibodies against TPO enzyme is important in making decisions about therapy, and should, therefore, be resolved in women with unusual thyroid function tests (Garber et al. 2012).

2.6.2 Hyperthyroidism (Thyrotoxicosis)

Another name used to describe hyperthyroidism is an overactive thyroid gland, characterized by increased thyroid hormone synthesis and secretion from the thyroid gland, resulting in accelerated metabolism in peripheral tissues. TSH levels are low while T_3 and T_4 levels are elevated. Thyrotoxicosis is a clinical syndrome and condition characterize by excessive circulation of thyroid hormones, T_4 and T_3 irrespective of the source. Other causes of thyrotoxicosis include thyroiditis, iodine-induced and drug-induced thyroid dysfunction, and factitious ingestion of excess thyroid hormones. The commonest cause of hyperthyroidism in the population worldwide is Graves' disease, followed by toxic nodular goitre. Graves' disease also called Basedow's disease, toxic diffuse goitre is usually of an auto-immune etiology that affect the thyroid gland (Patil-Sisodia et al. 2009). It is associated with eye disease (Graves' opthalmopathy) with a prevalence of 0.5% in males and 3% in females (Burch et al. 2012). Grave's disease is caused by thyroid-stimulating Immunoglobulin (TSI), an immunoglobulin G that mimics the action of thyroid stimulating hormone via cyclic AMP production. TSI has agonist properties, hence blocking TSH receptor not to respond to the feedback mechanism and thus continuously stimulate secretion of thyroid hormones T_4 and T_3. Hyperthyroidism can also be caused by toxic thyroid nodules (multinodular) and Sub-acute thyroiditis, caused by viral infection that prompt excess thyroid hormones production, abnormal secretion of TSH, excessive iodine intake, excess thyroid hormone tablets consumption and toxic thyroid adenoma / follicular adenoma ('hot' autonomous nodules) caused by low level of dietary iodine (Patil-Sisodia et al. 2009). Common hyperthyroidism clinical features include but not limited to irritability, weight loss**,**

palpitation, heat intolerance, opthalmopathy, diarrhea, and goitre. (www.niddk.nih.gov,2016). Last accessed on 2020, march 13.

Diagnosis

High thyroxine levels of T_4, T_3 and low TSH.

A high radioactive Iodine uptake is consistent with Grave's disease.

Detection of TSH-receptor antibodies (thyroid stimulating Immunoglobulin) are found in hyperthyroidism caused by Graves' disease (Gharib et al. 2010).

Treatment: Beta blockers to control rapid heart rate, anxiety and sweating. Auto thyroid medication against excess T_4 & T_3. Radioactive Iodine treatment to destroy part of thyroid gland and also surgery (Gharib et al. 2010).

Sub-clinical hyperthyroidism

These are individuals with overt primary hyperthyroidism, thyrotropin (TSH) hormonal levels are low or undetectable but T_4 and T_3 levels are raised. Subclinical hyperthyroidism is clinically a milder form of hyperthyroidism marked by low or undetectable serum thyrotropin levels, but with a normal serum thyroxine level (Vissenberg et al. 2012). Treatment of elderly people with subclinical hyperthyroidism could lower the frequency and the number of cases with arrhythmia (Negro et al. 2014). In persons diagnosed with subclinical hyperthyroidism, there is an increased risks of bone fractures and breakage by 42%. There is no evidence to support medical idea that treatment intervention for persons suffering from subclinical hyperthyroidism with anti-thyroid medications would reduce the risk of bone fractures. The introduction of assays for serum TSH sensitive enough to distinguish between normal and low concentrations allowed subjects with subclinical

hyperthyroidism to be identified. Subclinical hyperthyroidism is defined as a low-serum TSH concentration and normal serum T_4 and T_3 concentrations, without related hypothalamic or pituitary disorders / disease, non-thyroidal illness or ingestion of drugs that inhibit TSH secretion such as glucocorticoids or dopamine (Vanderpump et al. 2011).

2.6.3 Graves' Disease

Graves' disease, named after Robert J. Graves, MD, is a disease of an autoimmune type marked by thyrotoxicosis brought about by autoantibodies in blood circulation. This medical condition is caused by thyroid-stimulating immunoglobulins (TSIs) which has the same agonistic effect as thyrotropin hormone. TSI acts by binding to and activate thyroid stimulating hormone receptors (TSHr), causing the thyroid gland to proliferate and the thyroid follicular cells to increase the synthesis of thyroxine. Graves' disease, along with Hashimoto thyroiditis, is classified as an autoimmune thyroid disorder (Smith et al. 2016).

Pathophysiology: In Graves' disease, B and T lymphocyte-mediated autoimmunity are known to be directed at four main thyroid antigenic proteins namely: thyroglobulin protein, thyroid peroxidase enzyme, sodium-iodide symporter and the thyroid stimulating hormone receptor (TSHr). Nevertheless, among these four thyroid antigens, the thyroid stimulating hormone receptor itself is the key and primary auto-antigen of Graves' disease and is the cause of thyrotoxicosis manifestation. In Graves' disease manifestation, the antibodies and T-cell-mediated thyroid antigen-specific immune responses are well defined. Evidence of an autoimmune disorder mediated by reactive autoantibodies is the development and manifestation of thyrotoxicosis in healthy individuals by the transfer of thyroid stimulating hormone receptor antibodies

in serum from patients with Graves' disease and the passive transfer TSH receptor antibodies to the fetus during pregnancy (Bahn et al. 2010).

The thyroid gland is constantly stimulated by the circulating thyroid stimulating immunoglobulins directed at the TSHr, and pituitary thyrotropin secretion / release is inhibited due to the elevated production of thyroid hormones. Thyrotropin receptor antibodies stimulating activity belongs to the immunoglobulin G1 subclass. These thyroid-stimulating immunoglobulins (TSI) accelerate the release of thyroxine hormone and thyroglobulin protein and the pathway is mediated by 3,'5'-cyclic adenosine monophosphate (cyclic AMP), which also stimulate and activate iodine uptake, protein synthesis, and thyroid gland growth and proliferation (Yu et al. 2015). Conversion of peripheral blood mononuclear cell into $CD34^{+}$ fibrocytes is higher in Graves' disease than in healthy control individuals. These antigenic presenting cells ($CD34^{+}$ fibrocytes) contribute significantly to the pathophysiology of opthalmopathy by accumulating in orbital tissues and producing inflammatory cytokines, including TNF-alpha and IL-6 (Douglas et al. 2010). The most common cause of thyrotoxicosis in United States and developed countries is Graves' disease. Prevalence of maternal thyrotoxicosis in United State is 0.002%, with maternal Graves' disease being the commonest predominant etiology. Frequently, the affected patients have a family history involving a wide scale of autoimmune thyroid diseases, such as Graves' disease, Hashimoto thyroiditis, or post-partum thyroiditis, among others (McLeod et al. 2012). Among the causes of spontaneous thyrotoxicosis, Graves' disease is the most common. Graves' disease represents 60-90% of all causes of thyrotoxicosis in different regions of the world. If left untreated, Graves' disease can cause severe thyrotoxicosis, a life-threatening thyrotoxic crisis also known as 'thyroid storm' (exaggerated state of thyrotoxicosis), can arise. Persistent and prolonged form of

severe thyrotoxicosis give rise to severe weight loss with destruction of bones and muscles through catabolism (De Leo et al. 2016). Complications brought about by cardiac and psychocognitive can result in significant morbidity. Graves' disease is also associated with opthalmopathy, dermopathy, and acropachy. Protracted and prolonged excess secretion of thyroid hormone can results in osteoporosis in both men and women. The effect of bone loss and osteoporosis in women is particularly devastating, in whom the disease may worsen due to bone loss secondary to chronic anovulation or menopause. Bone loss is increase in patients with thyrotoxicosis. The marked increase and escalated bone loss can be illustrated by raised urinary pyridinoline cross-link excretion. Plasma FGF-23, inorganic serum calcium and phosphate cations, are significantly higher in the patients with Graves' disease compare to healthy control individuals, demonstrating that FGF-23 is physiologically associated to serum phosphate homeostasis mechanism in untreated Graves' disease subjects. About 25 to 80% of people with Graves' disease progress to developing eye problems (Brent et al. 2008). Advanced opthalmopathy can progress to compromised vision and blindness. Visual loss due to corneal lesions or optic nerve compression can be seen in severe Graves' opthalmopathy.

Graves' thyrotoxicosis accelerates muscular energy wastage and muscle protein catabolism. These abnormal conditions observe may explain the sarcopenia and myopathy exhibited in patients with thyrotoxic Graves' disease. Susceptibility is increased in females as with most common autoimmune diseases (Dong et al. 2014). Hyperthyroidism due to Graves' disease has a female-to-male ratio of 8:1. Typically, Graves' disease is a disease of young women, but it may occur in persons of any age. The typical age range is 20-40 years. Most affected women are aged 30-60 years age (Nikiforov et al. 2012)

2.6.4 Thyroid Nodules:

Nodules are lumps in the thyroid gland and most are harmless. Occasionally, normal thyroid gland tissue start to grow, causing one or several nodules to form. A solitary thyroid nodule is an abnormal growth (lump / mass) of thyroid cells or in one section of the thyroid gland and can cause swallowing or breathing problems. Thyroid carcinoma is the biggest concern when thyroid nodules form, but luckily the chances of it happening is low. The main cause is not known and undetermined, however, thyroid nodules and thyroid enlargement are frequently common in females than males and prevalence increases with age. Females commonly develop thyroid enlargement during gestation period and in menopause. There are different types of thyroid nodules and the most common types are:

Single thyroid nodule (Solitary nodule): It is a single palpable nodule in thyroid gland on clinical examination in an otherwise normal gland. There are two types: Toxic solitary nodule and non-toxic solitary nodule. Hot nodules means autonomous toxic nodule and normal nodule means one nodule and its surrounding tissue is inactive, therefore will not take up isotope. Overactive and warm nodule means one nodule and surrounding tissue will take up isotope. Cold non-functioning nodule means one nodule will not take up isotope. The presence of thyroid nodule in children and the aged can be cancerous. Deviation of the tracheal towards opposite side is frequent and is confirmed by trail sign, finger test and neck X-ray. The commonest site of a nodule is at the junction of isthmus with one of the lateral lobes.

Clinical features: Solitary nodule visible and palpable in one or adjacent lobes of the thyroid gland is normally smooth and firm. Single nodules are typically non-cancerous and usually can be left unattended. If a cancer is suspected by investigations, surgical intervention is recommended. Hot nodules (i.e. it produces too

much thyroid hormone), treatment is either by drug therapy, radioactive iodine or surgery.

Colloid (Solid) and hyperplastic nodules: These are one or more overgrowths of normal thyroid tissue. These growths are benign lumps (not cancer) which can be solitary or found in a multinodular goitre. They may grow big, but they don't spread beyond the thyroid gland. These nodules are usually observed without the need for surgical intervention.

Thyroid cysts (Cystic nodules): These are growths or swelling that contains fluid or blood or partly solid and partly fluid and can be managed by aspirating the fluid using a needle (fine needle aspiration). Surgical treatment is done in case fine needle aspirate procedure fails.

Thyroid adenoma: This is a benign and non-cancerous swelling of the thyroid gland, that is either inactive or active (functioning autonomously) as a toxic thyroid adenoma. It is difficult to differentiate from a cancer by scans and biopsy. It is commonly removed surgically so that its benign nature can be confirmed by close observation under the microscope (histologically). A thyroid adenoma is distinguished from a multinodular goitre of the thyroid in that an adenoma is typically solitary.

Inflammatory nodules: These nodules grow due to chronic (long-term) inflammation (swelling) of the thyroid gland and these growths can either cause pain or not.

Multi-nodular goitre: Sometimes an enlarged thyroid (goitre) is made up of many nodules (which are usually benign). A multinodular goitre is frequent and normally does not need an operation unless it causes swallowing problems, dyspnea or if the goitre is unsighted. It is uncommon to find cancer in a multinodular goitre. If the

thyroid gland is growing at a fast rate or one or more nodules suspected to be cancerous following investigation, then surgery is recommended.

Retrosternal Goitre: At times**,** a multinodular thyroid goitre grows down behind the breastbone causing constriction or squeezing the trachea and the large veins in the neck or the esophagus since it is in a fixed bony space. In this condition, surgery may be contemplated. The majority of the retrosternal goitres grow gradually over many years.

Hyper functioning thyroid nodules:

These are nodules which independently produce thyroid hormone without control of normal feedback mechanisms, leading to the development of hyperthyroidism. Uncontrolled hyperthyroidism exerts influence on the heart and leads to sudden cardiac arrest, raised blood pressure, arrhythmias, osteoporosis and other health related problems.

Thyroid cancer: Below 5% of the thyroid nodules are malignant. Patients with thyroid nodules are usually evaluated by Radio iodine or technetium 99 to determine whether thyroid tumour is solitary or multiple and whether it is hyper functioning or hypo functioning (‘hot’ or ‘cold ‘respectively). Patients with Solitary ‘cold’ nodule have 20% chance of the nodule being malignancy while ‘hot’ nodule is just hyperactive and benign

Diagnosis: Ultra-sound, CT scan or MRI. FNA biopsy to determine whether the nodule is cancerous (Braverman et al. 2012).

Treatment: Is based on the cause.

2.6.5 Thyroiditis:

Inflammation or swelling of the thyroid gland is commonly known as thyroiditis. Thyroiditis is a group of thyroid disorders that all give rise to thyroidal inflammation. Categories of the thyroiditis disease include: Hashimoto's thyroiditis, (most common cause of hypothyroidism in the USA), postpartum thyroiditis, sub-acute thyroiditis, silent thyroiditis, drug-induced thyroiditis, radiation-induced thyroiditis, acute thyroiditis, and Riedel's thyroiditis (Agrawal et al. 2016)

Thyroiditis come about as a results of an attack on the thyroid gland, leading to inflammation and damage to the thyroidal cells. This disease is thought about to arise due to a malfunction of the immune system. Antibodies that attack the thyroid gland are the cause of most types of thyroiditis. Thyroiditis can also be caused by an infection caused by viruses or bacteria, which are directed to work in the same way as antibodies to produce inflammation in the thyroid glands (Maati et al. 2011). Sub-acute granulomatous thyroiditis (painful) is due to viral infection and responsible for about 5% of the visits to physicians due to thyroid abnormalities. It is associated with symptoms of hyper metabolism such as diaphoresis, tachycardia, palpitation and weight loss when there is co-existent hyperthyroidism and contribute 5 – 20% of all patients with thyrotoxicosis. Sub-acute lymphocytic thyroiditis (painless) occurs sporadically or in post-partum and commonly presents as hypothyroidism. Some people produce thyroid antibodies, and thyroiditis can be deemed as an autoimmune disease, because the body acts as if the thyroid gland is not self. Certain drugs, like interferon and amiadorone, can also cause thyroiditis because they have a tendency to damage thyroid cells.

Diagnosis

The most common method to diagnose thyroiditis is to palpate the thyroid gland during a physical examination. Evaluating the patient for elevated levels of erythrocyte sedimentation rates (ESR), elevated thyroglobulin levels, and depressed radioactive iodine intake.

Measuring the levels of thyroid stimulating hormone the pituitary gland is producing and the type of antibodies present in blood. In some cases FNA for cytology is taken to aid in arriving at diagnosis. Majority types of thyroiditis are three to five times more apparently to be found in females than in males. The average age of onset is between 30 - 50 years of age (Sweeney et al. 2014).

Treatment: Treatments depend on the type of thyroiditis that is diagnosed. For the most common type, Hashimoto's thyroiditis, the treatment is hormone replacement to prevent or corrects the hypothyroidism. Non-steroidal anti-inflammatory medications to reduce inflammation and to control palpitations. Prescription of beta blockers to lower the heart rate and reduce tremors until the initial hyperthyroid period is resolved (Bindra et al. 2006; De Leo et al. 2016).

2.6.6 Thyroid cancer

Thyroid cancer is a rare cancer of the endocrine which develops within the tissues of the thyroid gland. Common clinical presentation include enlargement or a lump in the neck. Thyroid cancer is a disease with abnormal cells' growth and has the potential to metastases to other parts of the body (Andresen et al. 2017). Thyroid cancers are classified according to their histopathological characteristics into four main types: papillary CA, follicular CA, medullary CA and anaplastic CA. Thyroid cancers are considered to be linked to a number of environmental radiation, exposures at a young

age and genetic predisposing factors. Genetic causes include, family history and multiple endocrine neoplasia type 2 which increases rates, particularly of the rare medullary form of the disease (Vecchia et al. 2015). Thyroiditis and some thyroid diseases can lead to the development of thyroid cancer (Pacini et al. 2012). Thyroid cancers are relatively rare form of malignancy worldwide. They are however noted to be the commonest occurring endocrine malignancy. Ninety percent of thyroid cancers (TC) occurring worldwide are well-differentiated types and include follicular and papillary cancers which also have favourable prognosis than the medullary and anaplastic which are undifferentiated (Gharib et al. 2010). Women develop papillary cancer three times more frequent than men do, and the mean age of presentation is 30-40 years (Repplinger et al. 2008). It has been shown that high prevalence of anaplastic cancers occurred in the same regions as with follicular cancers. Medullary carcinoma of the thyroid is a distinct thyroid carcinoma originating in the parafollicular cells of the thyroid gland and is the least documented malignancy (Pappa et al. 2016).

Diagnosis: Thyroid ultrasound, fine needle aspiration for cytology / histopathology technique (Andresen et al. 2017).

Treatment: Include surgery, radiation therapy (radioactive iodine), chemotherapy, thyroid hormone targeted therapy (Andresen et al. 2017)

2.6.7 Goitre

Goitre is a swelling in the neck resulting from an enlarged thyroid gland (thyromegally), associated with thyroid gland that is not functioning properly. Worldwide, over 90% cases of goitre are caused by iodine deficiency (Carle et al. 2014). Other types of goitres are:

Colloid goitre (Endemic). A colloid goitre / endemic goitre develops due to lack of iodine, a trace mineral fundamental for the synthesis of thyroid hormones. Toxic nodular or multi-nodular Goitre, Hashimoto's disease, Graves' disease, thyroid nodules or single nodule which develops in one part of the thyroid gland, Most nodules are non- cancerous, benign and do not lead to cancer, iodine deficiency, familial or non-toxic sporadic MNG, follicular adenoma, thyroiditis (inflammation) and thyroid cancer.

Goitre is classified into the following classes according to growth pattern:

1. **Simple Goitre** (Euthyroid) – Enlargement of thyroid gland without toxic manifestation.

Causes:

i) Iodine deficiency

ii) Absolute deficiency-Areas far from sea.

iii) Relative deficiency-Due to increase demands for iodine at pregnancy, puberty and lactation (physiological)

Dyshormogenesis-Hereditary deficiency of enzymes necessary for thyroxine formation.

Ingestion of goitrogens-High calcium, cabbages, cauliflowers which contains thiocyanate which inhibits iodide transport within the thyroid (NIS).

Types of simple Goitres

a) Simple diffuse hyperplastic- physiological, pubertal, pregnancy.

b) Simple colloid goitre.

c) Simple nodular goitre.

2. **Toxic Goitre**

a) Diffuse (Graves' disease) – Primary toxic goitre

b) Multi-nodular – Secondary toxic goitre

c) Toxic adenoma (Toxic solitary nodules)

3. **Neoplastic**- Benign and Malignant

4. **Inflammatory**- Autoimmune, granulomatous, fibrosis and infective.

Types of Goitre

i) **Functional:**

Euthyroid goitre – Diffuse and nodular goitres.

Hypothyroid goitre – Endemic goitre (IDD) where more than 10% of the population is affected, Hashimoto's disease.

Thyrotoxic (hyperthyroid) goitre – Graves' disease, Toxic multi-nodular goitre, Toxic Uni-nodular goitre (Solitary nodular goitre).

ii) **Structural**: Diffuse (colloid) and Nodular goitres.

Pathophysiology of Goitres

Uni-nodular / Solitary nodular goitre: One thyroid nodule can be either inactive or toxic.

Multi-nodular goitre (multiple nodules), or multinodular goitre also known as Plummer's disease, can be inactive (non- toxic) or toxic (toxic multi-nodular) and is associated with hyperthyroidism found in 13.7% of patients with multi-nodular goitre (Sturniolo et al. 2013).

Formation of multi-nodular goitre undergoes the following developmental stages, namely: stage of goitre hypertrophy and hyperplasia, stage of thyroid stimulating hormone fluctuation and finally the stage of formation of nodules (inactive).

Clinical features of Multi-nodular goitre: Multi-nodular goitre is common and established in middle aged women (40 – 60 years). It is slow and progressive disease with many years of natural history. Many nodules of various sizes are formed in both lobes, in isthmus, which is harden, non-tender, nodular, moves with deglutition and an increase in size may signifies malignant transformation or hemorrhage. These nodules secrete thyroid hormones autonomously, suppressing TSH-dependent growth and function in the rest of the gland. Graves' disease is the leading cause of hyperthyroidism in the developed world, followed by toxic multinodular goitre, while on the other hand, iodine deficiency is the commonest cause of hypothyroidism in the developing-world, where the concern population suffer from iodine deficiency (reduce iodine leads to decline thyroid hormone synthesis). However, iodine deficiency can cause goitre (thyroid enlargement); within a goitre, nodules can develop. Risk factors for toxic multinodular goitre include individuals over 60 years of age and being female (Vanderpump et al. 2011).

Causes

a) Lack of trace element iodine leading to reduce T_4 production / synthesis

b) Introduction of thyroid cell hyperplasia precipitated by low levels of T_4, leading to the appearance of multinodular goitre.

c) Escalation in the replication process, can results in susceptibility to risk of mutation in the TSH receptor.

Constitutively active and mutated thyrotropin receptor (TSHr) would eventually become toxic leading to the production of excess and harmful T_3 / T_4 resulting in hyperthyroidism.

iii) **Diffuse goitre:** The whole thyroid gland is generalized enlarged. Diffuse toxic goitre is also known as Graves' disease and is the most common cause of hyperthyroidism (over activity of the thyroid gland), with generalized diffuse over activity ("toxicity") of the entire thyroid gland which becomes enlarged into a goitre. In diffuse toxic goitre, the thyroid gland is abnormally increases in size and spread, hence becoming hyperplastic and eventually produces excess thyroid hormone. This leads to increase and accelerated metabolism in the majority of the body's tissues. The clinical effects and its presentations are varying in distribution, intensity and are modified by age, gender, and related medical problems. Graves' disease is often applied when diffuse toxic goitre is associated with clinical evidence of oculopathy / opthalmopathy or rarely with dermopathy. Diffuse toxic goitre and its hyperthyroidism are caused by TSH-receptor stimulating antibodies on the thyroid follicular cells. These antibodies stimulates iodine uptake, thyroid hormonogenesis and release, and thyroid gland growth. The exact cause of TSH- receptor stimulating antibodies presence on the thyroid follicular cells is not well understood, however some authorities have suggested that there is a genetic predisposing factor leading to lack of suppressor T cells. The absence of suppressor T-cells results in the unregulated production of the antibodies which lead to the occurrence of autoimmune disease. These antibodies may cross the placenta resulting in fetal and neonatal hyperthyroidism. The production of thyrotropin receptor antibodies are mainly confined within the thyroid gland, they however reach circulation and can be assayed by various methods. Diffuse toxic goitre is mostly common in females than males,

giving female to male ratio of 8:1. It is frequently associated with or following pregnancy. Diffuse toxic goitre is uncommon in children below 10 years and rare in aged persons but it can occur in persons of all ages. The peak incidence of occurrence is in third and fourth decades of life. Frequency is increased in postnatal women, when the initial presentation of disease normally occurs. Associated opthalmopathy is not well understood, but it is a related but separate autoimmune disorder directed toward the extra ocular muscles. If an associated opthalmopathy is present, the diagnosis of diffuse toxic goitre is obvious.

iv) **Endemic goitre**: Is associated with dietary iodine deficiency. In this condition, thyroid hormones (T_4 & T_3) synthesis is impaired by iodine deficiency, plasma T_4 and T_3 concentrations fall hence increasing TSH secretion.

v) **Euthyroid / Simple goitre:** Thyroxine synthesis may be impaired by iodine deficiency, drugs such as para-amino salicylic acid or enzyme deficiency. The tendency of the plasma T_4 concentration to fall increases TSH secretion. The thyroid cells synthesis and secrete T_4 and T_3 eventually becoming hyperplastic resulting in thyroid enlargement as a compensating mechanism. T_4 and T_3 plasma levels are maintained at the expense of the development of goitre (Gupta et al. 2015).

Diagnosis of goitres

i) Measuring the levels of T_4, T_3, TSH and thyroid auto-antibodies.

ii) Ultra-sound of the thyroid gland to check swelling or nodules and Radioactive Iodine uptake (RAIU) (Haugen et al. 2016)

Treatment: Surgery and Radio-active Iodine (RAI) (Yener Ozturk et al. 2014).

2.6.8 Iodine deficiency disorders (IDD)

Deficiency diseases can be defined as pathological states with characteristic clinical signs, which are due to deficiency in the diet of a nutrient and can be prevented or cured by providing the missing nutrient. The WHO defined IDDs as entire consequences as a results of iodine deficiency in a population and can be corrected by ensuring adequate intake of iodine in the diet. Inadequate iodine intake in pregnancy and infancy can give rise to neurological and psychological complications in children. Children living in severely iodine-deficient areas have lower intelligence quotient (IQ) than those children living in iodine-replete areas (de Escobar et al. 2007). Iodine deficiency is the foremost, and major cause of avoidable mental retardation worldwide (Zimmermann et al. 2015). Worldwide, the iodine status of the majority of premature infants shows inadequate iodine (Belfort et al. 2012). In a prospective and longitudinal study, continuous decreases in TSH and increases in T_4 were observed in a formerly iodine deficient population, even after iodine correction and supplementation in the presence status, suggesting that decrease iodine intake at tender age results in thyroid autonomy that persists in spite of normal iodide intake later in life (van de Ven et al. 2014). At present, approximately, a third of the world's population reside in regions of inadequate dietary iodine levels making iodine-deficiency the most common cause of hypothyroidism and endemic goitre (Burch et al. 2012). The prevalence of goitre is as high as 80%, in areas of extreme iodine deficiency, whereas on the other hand in areas where iodine-deficiency is not found, the commonest type of hypothyroidism is an autoimmune condition called Hashimoto's thyroiditis, with a prevalence of 1-2% (Ross et al. 2016). The commonest cause of thyroid disorders in endemic countries is iodine deficiency which is a major public health problem in the affected countries. Worldwide, 2.2 billion

persons are at risk for iodine deficiency disorder and related complications and it is estimated that 30-70% have goitre and 1-10% have cretinism (De Benoist et al. 2008). Approximately, 8% of newborns from sub-Saharan Africa are at risk of mental retardation resulting from iodine deficiency related disorders (Kishosha et al. 2011). The range of iodine deficiency disorders complications in young people include: goitre, clinical and subclinical hypothyroidism, cognitive impairment, cretinism, and increased vulnerability of the thyroid gland to nuclear radiation (Kishosha et al. 2011). In adults, IDD include goitre with its complications, hypothyroidism and impaired mental function, spontaneous hyperthyroidism in the elderly and iodine-induced hyperthyroidism. Endemic goitre is a predominant feature of iodine deficiency. Goitrogens (substances that hinders the functioning of the thyroid gland by impeding the uptake of iodine) also play a major role in the development of endemic goitre. In Africa, goitrogens include thiocyanate usually found in poorly cooked cassava, a source of carbohydrate and staple food commonly eaten in many African communities. Selenium deficiency is also reported as a contributory factor in the occurrence of endemic goitre in Africa even after correction for iodine deficiency (Kishosha et al. 2011). Some studies have found that the prevalence of thiocyanate overload and iodine deficiency to be 20% and 21%, respectively (Taga et al. 2008). Endemic cretinism which happen to be found in areas of acute iodine deficiency, manifest itself by myxedematous form which is the commonest occurring neurological form of cretinism in Africa continent and its prevalence rate ranges from 1.2% to 6% with Central Africa recording the highest rate (Um Sap et al. 2015).

2.6.9 Autoimmune thyroid disease

Autoimmune thyroid disease (AITD) is the commonest autoimmune disorder resulting in either hyperthyroidism or hypothyroidism. Auto-immune thyroid disease is a chronic disease in which the body interprets/recognizes or identifies the thyroid gland and its hormone products T_3, T_4, and TSH as foreign, therefore producing special antibodies that target the thyroid cells, thereby destroying it. AITD presents with Hyperthyroidism (in which Grave's disease is the commonest) and Hypothyroidism with or without goitre. Graves' disease is the predominant AITD globally (Skarpa et al. 2011).

In African countries it was shown that the universal incidence of AITD in Tunisia was 9.9% and this was noted to have occurred in concurrence with 6.3% of other autoimmune disease (Ogbera et al. 2011). In Ethiopia, the prevalence of autoimmune thyroid disease was reported to be between 1.2% and 3.7% (Ogbera et al. 2011). The low prevalence of the reported cases of auto-immune thyroid diseases in Africa maybe due to financial constraints and missed diagnoses among other factors. In South Africa, it was found that unusual features and complications of thyrotoxicosis e.g. cardiac complications myopathy and invasive eye diseases were noted to affect and occur more in black South Africans than the whites (Skarpa et al. 2011). In Togo, cardiac complications were documented in 46.6% of patients with thyrotoxicosis and smaller frequency rate of 12.6% was documented for phytotoxic heart disease in DR Congo (Skarpa et al. 2011). A study in Lagos, Nigeria showed that 42% of subjects with thyrotoxicosis had an occurrence of heart failure (Ogbera et al. 2011). Other common autoimmune diseases of the thyroid gland are mostly under diagnosed and under reported (Cappa et al. 2010). Studies on genetic and environmental factors have shown an association between Human lymphocytic Antigen (HLA) classes II

molecules (DR3, DR4 and DR5) and the incidence of Hashimoto's thyroiditis. The cytotoxic T cell (pathway) surface molecule may have a role in Hashimoto's thyroiditis predisposing environmental factors (smoking and high iodine intake) have been found to be linked to increase and higher incidence of AITD. The immunological process triggered by thyroid peroxidase enzyme is demonstrated in the patient's antibody status. It has been shown that the prevalence of thyroid peroxidase (TPO) and thyroglobulin (Tg) antibodies increases with increasing age, and that the prevalence of TPO antibodies is higher in all age groups than that of thyroglobulin antibodies (Pedersen et al. 2011). Studies have shown that women over 20 years are more likely to produce thyroid antibodies than men (Diana et al. 2017). The extent of antibody profiles in cases of suspected AITD in Africa is obscured given the availability of only few studies on thyroid disease in the continent. Majority of the countries in Africa are poor and lack resources, hence described as "resource poor" and great attention of management of AITD is treating clinical presentation, and not carrying out thorough and comprehensive investigations (Ogbera et al. 2011). Even though thyroid peroxidase antibodies (TPOAb) and / or thyroglobulin antibodies (TgAB) are regularly exist in the sera of patients with AITD, some patients often have negative thyroid autoantibody test results. Thyroid receptors antibodies (TSHRAb) or Thyroid stimulating immunoglobulin (TSI) are present in most patients with a history of or who currently have Graves′ disease. The clinical significance of these antibodies in the African context is grossly understudied and their usefulness may lie in their being highly suggestive of autoimmune diseases. Studies on thyroid antibody profile show TPO antibody to be the commonly detected antibody in autoimmune thyroid disorders. Other studies have also shown that tuberculosis can also cause thyroid dysfunctions (Führer et al. 2012). Hashimoto' thyroiditis has also been reported to be

the leading cause of hypothyroidism. The treatment of thyroid disorders (cancer) using radioactive iodine also predispose and leads to hypothyroidism (Fualal et al. 2012).

2.7 Management of thyroid disorders

Fully equipped diagnostic and investigation facilities for thyroid disorders / disease are absence in the majority of countries in African continent and the routinely employed diagnostic and investigative techniques include immunoassays, serology, ultrasonography, cytology, and histopathological techniques for the overall evaluation of thyroid diseases (Wu et al. 2016). Advanced techniques e.g. computed tomographic scans and magnetic resonance imaging facilities are extensively not available but when available are inaccessible for most patients because of the system of health care provision which is often that of "out of pocket" payment. Fine needle aspiration for cytology (FNAC) is commonly employed in the evaluation of thyroid nodules in the African continent (Rong et al. 2016). The mode of treatment that are often employed in the management of thyrotoxicosis are pharmacotherapy (thionamides) and surgery in goitre / thyroid nodules (Guan et al. 2017)

CHAPTER THREE

3.0 METHODS

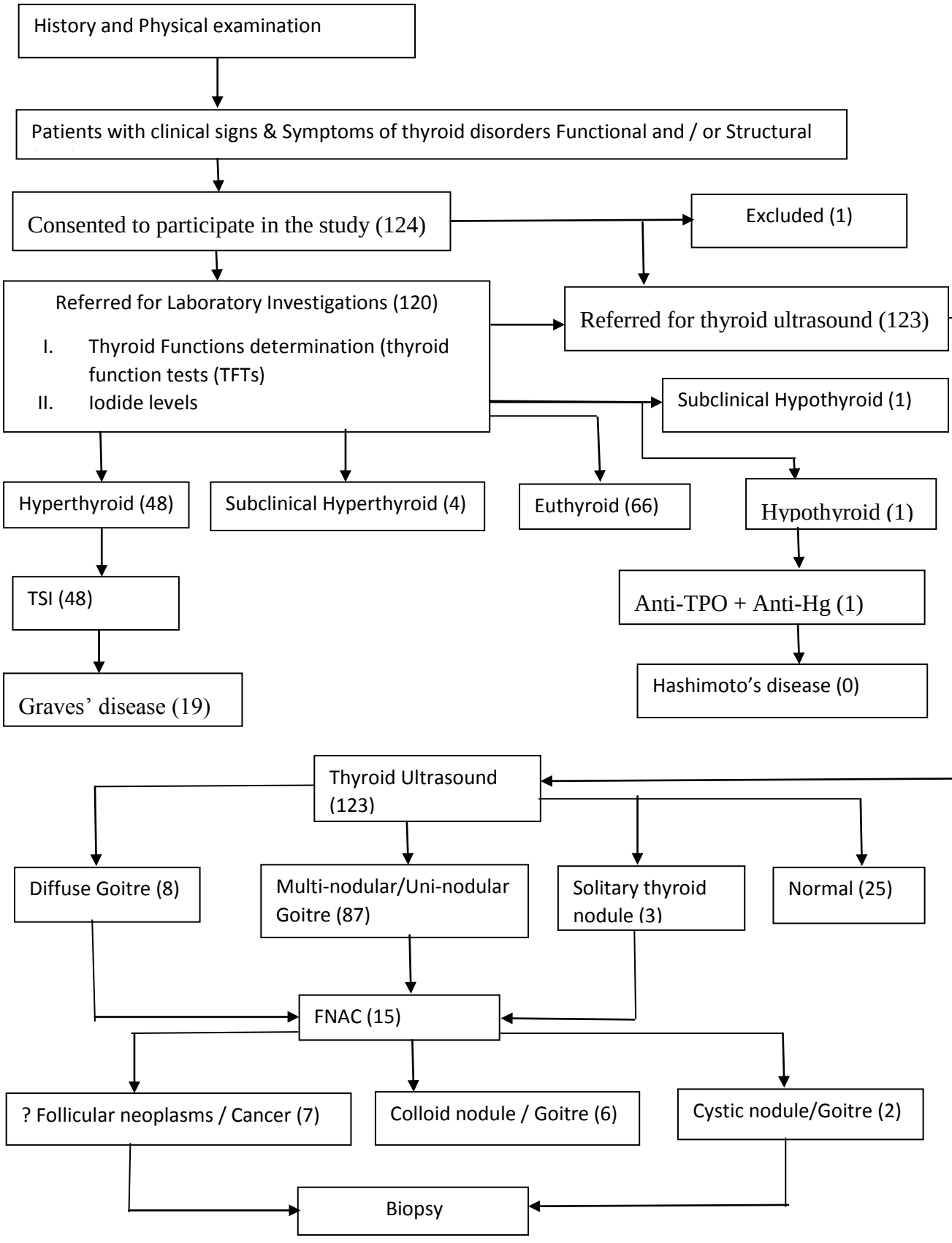
3.1 Study site

The study was conducted at Nakuru level 5 Hospital surgical clinic between 4th February, 2018 and 28th June, 2018. Nakuru level 5 Hospital is the largest regional Referral Hospital for eight (8) counties (Nakuru, Bomet, Baringo, Narok, Kericho, Samburu, Laikipia and Nyandarua) and serving a population of about 3.6 million. The hospital has a bed capacity of 500 with bed occupancy of an average of 120%. It also serves as a teaching facility for Medical institutions.

3.2 Study design:

The study was a descriptive cross-sectional study of all patients presenting with thyroid disorders or clinical signs and symptoms of thyroid disorders. Patients were clinically evaluated for features of thyroid disorders. Sampling was done by census enrollment of all patients referred to the surgical clinic from the general outpatient department who during the study period fulfilled the eligibility criteria. Referrals from outside the tertiary healthcare facility are also seen first in general outpatient department.

3.2.1 Flow chart screening of study patients



KEY: ( ) = Number of Participants

3.3 Study population

The target population was all patients attending Nakuru level 5 Hospital surgical clinic with thyroid disorders or having clinical signs and symptoms of thyroid disorders with or without goitre, who were initially referred to the laboratory and /or radiology departments from general outpatient department between 4th February, 2018 and 28th June, 2018.

3.4 Definition of Cases

3.4.1 Inclusion Criteria:

i. All patients with clinical signs and symptoms of thyroid dysfunction e.g. easy fatigability, weight loss or gain, dry skin and hair, cold or heat intolerance, nervousness and palpitation referred to the laboratory and / or radiology departments for investigations.

ii. All patients presenting with thyroid disorders attending Nakuru level 5 Hospital, surgical clinic.

iii. Patients must consent to participate in the study.

3.4.2 Exclusion Criteria.

i. Patients with thyroid disorders or clinical signs and symptoms of thyroid disorders who were not referred to the laboratory and / or radiology departments for investigations.

ii. Patients who never consented to participate in the study.

iii. Patients with existing cases of thyroid disorders.

3.5 Data collection and management

3.5.1 Study procedure

After the Hospital Research & Ethics Committee approval was obtained, ref. RII/VOL.1/08 (appendix IV), a template in form of a structured standard questionnaire (appendix I) in English version designed based on the objectives of the study was used to capture data on basic information of patient's identity, clinical history and physical examination, signs and symptoms, demographic characteristics and risk associated factors. Each questionnaire clearly labelled with serial number was administered in privacy. Subsequently, blood samples were drawn for biochemical investigations / assays. Other additional investigations which were included were fine needle aspirate for cytology (FNAC) and thyroid ultrasound. Other clinical findings were obtained from the patient's records file to assist in arriving at a diagnosis. Results from the laboratory assays, radiological reports and cytological findings where applicable were entered using the same identification number as the one on the questionnaire.

3.5.2 Collection of blood Samples for Biochemical assays

Venous blood (5.0 mls) without anticoagulants was aseptically collected from each of the participants and allowed to clot. Serum was separated from clotted blood by centrifugation at 3000 rpm for 3 minutes, and sera pipetted out into Eppendoff tubes, labelled and numbered to correspond with the patient's details. Serum samples were then subjected to biochemical assays using automated Electrochemiluminescence (ECL)-Immuno-assay method using Cobas e 411 analyzer. Patients' samples not assayed were immediately frozen at -20^{o}C.

3.5.3 Laboratory analysis / Blood hormonal assays (Chemiluminescent Immuno-assay method)

Standard thyroid function tests (TFTs) were performed on patients' serum according to the manufacturer's instructions from ROCHE Dialog CO. LTD. The methods employ specific antibody reagents directed at the ligands of free hormone moieties of T_4, T_3 and TSH, Measurement of thyroid hormones levels TSH, T_4, T_3, anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies were performed on serum specimens by automated Electrochemiluminescence (ECL)-Immuno-assay method using Cobas e 411 analyzer, employing competitive principle for extremely small analytes of low molecular weight like T_4 and T_3. The method employs a combination of monoclonal dye conjugate of T_3 or T_4 antibodies coated on solid phase to identify T_3 or T_4 hormone moieties in the test sample with a high degree of specificity. The amount of light produced is inversely proportional to the concentrations of T_3 & T_4 in the sample. TSH a glycoprotein is measured using two steps sandwich principle for higher molecular weight analytes. This is a quantitative assay for the detection of TSH in serum for hypothyroidism or hyperthyroidism diagnosis. The method employs a combination of monoclonal dye conjugate and polyclonal-solid phase antibodies to identify TSH in the test sample with a high degree of specificity. In summary these methods employ specific antibody reagents directed at the ligands of free hormone moieties of T_4, T_3 and TSH.

3.5.4 Thyroid auto-antibodies assays (Chemiluminescent Immuno-assay method)

Thyroid peroxidase antibodies (TPOAb) assay is a sequential two-step Immuno-enzymatic (Sandwich) assay. A Sample is added to a reaction vessel with paramagnetic particles coated with Thyroid peroxidase protein. After incubation, materials bound to the solid phase are held in a magnetic field, while unbound

materials are washed away. The protein A-alkaline phosphatase conjugate is added and binds to the TPOAb. After the 2nd incubation, the reaction vessel is washed to remove unbound materials. A chemiluminescence substrate (Lumi-pho++ 530) is added to the reaction vessel and the light generated by the reaction is measured with a Luminometer. The light production is proportional to the concentration of TPO antibodies in the sample (Cinquanta et al., 2017)

Thyroglobulin antibody (TgAB) assay is a sequential two-step Immuno-enzymatic (Sandwich) assay. A Sample is added to a reaction vessel with paramagnetic particles coated with Thyroglobulin protein. After incubation, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. The thyroglobulin-alkaline phosphatase conjugate is added and binds to the TgAB. After the 2nd incubation, the reaction vessel is washed to remove unbound materials. A chemiluminescent substrate (Lumi-pho++ 530) is added to the reaction vessel and the light generated by the reaction is measured with a Luminometer. The light production is proportional to the concentration of Thyroglobulin antibodies in the sample (Cinquanta et al., 2017).

In Thyroid stimulating immunoglobulin assay (inhibition assay), a sample suspected to contain Thyroid receptor antibodies (TRAb) / Thyroid stimulating immunoglobulin (TSI) is added to a reaction vessel with Purified human recombinant TSHR coated (immobilized) on polystyrene tubes (solid phase). After incubation, materials bound to the solid phase are held in polystyrene tubes, while unbound materials are washed away. A chemiluminescent substrate labelled bovine TSH is added to the reaction vessel and the light generated by the reaction is measured with a Luminometer. The light production is inversely proportional to the concentration of thyroid stimulating

immunoglobulin (TSI) in the sample. This assay is based on the ability of TRAb (TSI) to inhibit TSHR binding by labelled bovine TSH.

Thyroid status was defined as follows based on the above biochemical assays:

I. Euthyroid TSH, T_4, T_3 and iodide (I^-) levels within their normal ranges, (0.2 - 5.2 µIU/L), (4.1 – 11.6 µg/dl), (1.1 – 4.6 ng/ml) and (5 - 18µg/dl) respectively.

II. Hypothyroid (TSH level >5.2 µIU/L, T_4 level < 4.1 µg/dl and I^- level < 5.0 µg/dl

III. Subclinical hypothyroid (TSH level >5.2 µIU/L, normal T_4, T_3 and I^- levels

IV. Hyperthyroid (TSH level ≤ 0.2 µIU/L, T_4 level > 11.6 µg/dl and I^- level > 18µg/dl

V. Subclinical hyperthyroid (TSH level, < 0.2 µIU/L) and normal T_4, T_3 and I^- levels

VI. Graves' disease: (TSI >1.5 IU/L)

VII. Hashimoto's disease: (Anti-TPO >35.0 IU/mls & Anti-Hg >20.0 IU/mls

3.5.5 Thyroid ultra-Sound Scanning

All the study patients referred to the radiology department, with or without goitre on physical examination, had ultrasound imaging of the thyroid gland done for the purpose of measuring the thyroid size. Ultrasound compliment information gained from history and physical examination. In addition it is very resourceful in investigating patients presenting with thyroid nodules to determine whether they are single or multiple.and the nature of nodules, either solid or cystic.

3.5.6 Fine needle aspirate for cytology

Fine needle aspirate was done on all patients with cystic nodules or cystic goitres that were suspicious of malignancy. Aspirate were taken using 21 gauge needle. Aspirates were air-dry for 20 minutes fixed in absolute ethanol and transported to the cytology

laboratory. In the laboratory, they are stained with Haematoxylin and Eosin or papanicolaou stains. Cytologist examined the slides to determine the nature of the goitre / cystic nodules.

3.5.7 Serum inorganic iodide determination:

Serum inorganic iodide was estimated quantitatively, according to modified method for determination of inorganic iodide in plasma / urine employing Iodometric titration Technique using standardized sodium thiosulphate and starch as an external indicator (appendix V). The end point of titration is the change of colour from dark-blue complex to clear colour. The principle of the reaction in this titration is the reduction of iodine with Sodium thiosulphate. salt (Modified, S. K. Giri et al..2007).

Principle of the Test procedure (Iodometric titration Technique)

Dichromate oxidizes iodide to iodine. The iodine liberated is then titrated with standardized sodium thiosulphate.

$$Cr_2O_7^{2-} + 6\,I^- + 14H^+ \longrightarrow 2Cr^{3+} + 3I_2 + 7H_2O$$

$$2S_2O_3^{2-} + I_2 \longrightarrow S_4O_6^{2-} + 2I^-$$

(Dark-blue) (Colourless)

3.5.8 Background information

The following information was collected in the questionnaire.

Clinical Profile

i) Chief complains – symptoms of the presenting complains eg.swelling / mass in the neck, Dysphagia, dyspnea, pain / tenderness in the neck etc.

ii) Clinical presentation e.g. goitre, pressure signs, exophthalmos, weight loss, tremors, sweats etc.

iii) Risk factors e.g. history of endocrine disease, familial endocrine disease, history of neck or head radiation, dietary intake (goitrogens) etc.

Investigations (Screening for thyroid disorders)

Biochemical assays:

i) T_4, T_3, & TSH

ii) TSI, Anti-TPOABs & Anti-TgAB

iii) Inorganic serum iodide level

Non-biochemical techniques:

i) Thyroid ultrasonography imaging

ii) Fine needle aspirate for cytology

iii) Histological examination (rare) for suspected cases of malignancy

3.5.9 Data analysis and presentation

All data were entered into Excel spreadsheet (MS Excel®, Microsoft USA) and subsequently, stored, cleaned and coding through Microsoft access. The available data was statistically performed and analyzed using STATA version 15 statistical software package, (Stata Corp, Texas). Descriptive statistical analysis was done to determine frequencies and percentages as well as mean and standard deviation (SD) of age. The data were presented in tables, graphs, percentages, charts and were used to summarize the distribution of various thyroid disorders, clinical presentations and demographic characteristics among the patients. This was done according to the following:

a) The proportion of patients who had specific thyroid disorders:

i. Goitres and their classifications based on growth patterns (structural and functional)

ii. Solitary thyroid nodule

iii. Graves' disease

iv. Thyroid cancers or malignancies

b) Proportion of patients with various clinical presentation / features common among patients with different thyroid disorders (abnormal thyroid function / thyroid dysfunctions).

c) The proportion of patients who had:

i. Clinical hyperthyroidism

ii. Clinical hypothyroidism

iii. Euthyroidism

iv. Subclinical hyperthyroidism

v. Subclinical hypothyroidism

The distribution of goitre cases among the patients were further classified as simple multi-nodular (non-toxic), toxic multi-nodular, simple diffuse, Euthyroid, cystic, recurrent multi-nodular, endemic multi-nodular and solitary / Uni-nodular. The relationship between thyroid disorders with demographic characteristics and clinical presentation were analyzed.

CHAPTER FOUR

4.0 RESULTS

A total of 174 patients were referred and evaluated at the surgical clinic with thyroid disorders / disease or clinical features of thyroid disease / disorders between 4th February, 2018 and 28th June, 2018. Of the 174 patients evaluated, 124 consented and were recruited for the study, while the rest were found not to have thyroid disease / disorders on evaluation or refused to consent to take part in the study and therefore were excluded. One case with existing thyroid disorder of multi-nodular goitre was excluded from laboratory and radiological investigations. This study sought to obtain the following set of data from the study participants: Demographic characteristics, clinical findings results as well as laboratory results for thyroid hormonal profile, thyroid auto-antibodies levels, iodide element levels, FNA biopsy as well as thyroid ultrasound results.

4.1 Socio-demographic characteristics of the patients

The following social demographic characteristics were determined among the study participants:

i) **Gender**

One hundred and fourteen (114) patients were females (92.0%), while ten (10) were males representing (8.0%) of the total participants, giving a female to male ratio of 11: 1 (**fig. 6**).

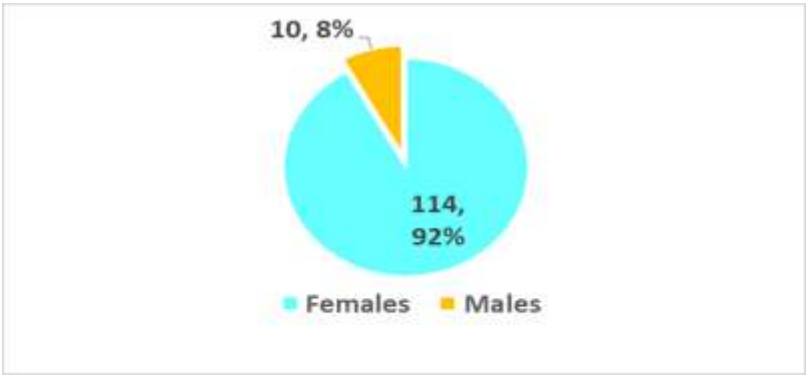


Figure 6 Pie chart Showing Gender of study patient

ii) Age

The age range of the patients studied was 3 months to 78 years, with a mean age of 40.5 ± 15.4 years. Fifty-four patients (43.5%) were aged between 21 years and 40 years and majority were female (96.3%). Two patients (1.6%) were below 5 years of age and among these, one patient with a history of familial hyperthyroidism was three months old. Most of the male patients were of medium age where the average age was 46.0 years and none of them was above 60 years or below 20 years of age. On the other hand, female patients were young with an average age of 40.0 years and 13 (10.5%) were aged above 60 years **(fig. 7**).

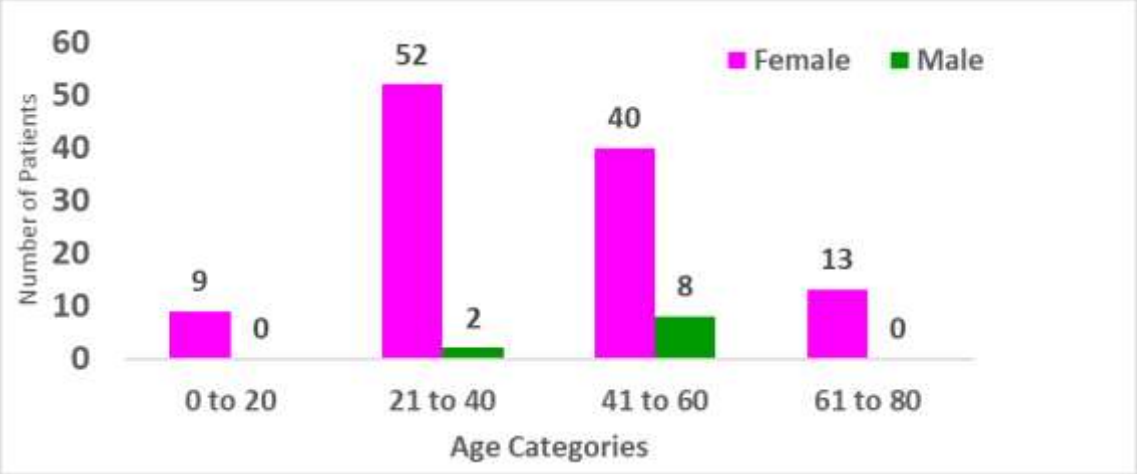


Figure 7: Distribution of patients by Age and Gender

iii) Region of Residence

The different regions of residence of the study participants were recorded and presented as in figure 8 below.

Majority of the patients, 105 (84.7%) resided in Nakuru County. Only a few of the patients 19, (15.3%) resided in other eight Counties of Kenya (**fig. 8**).

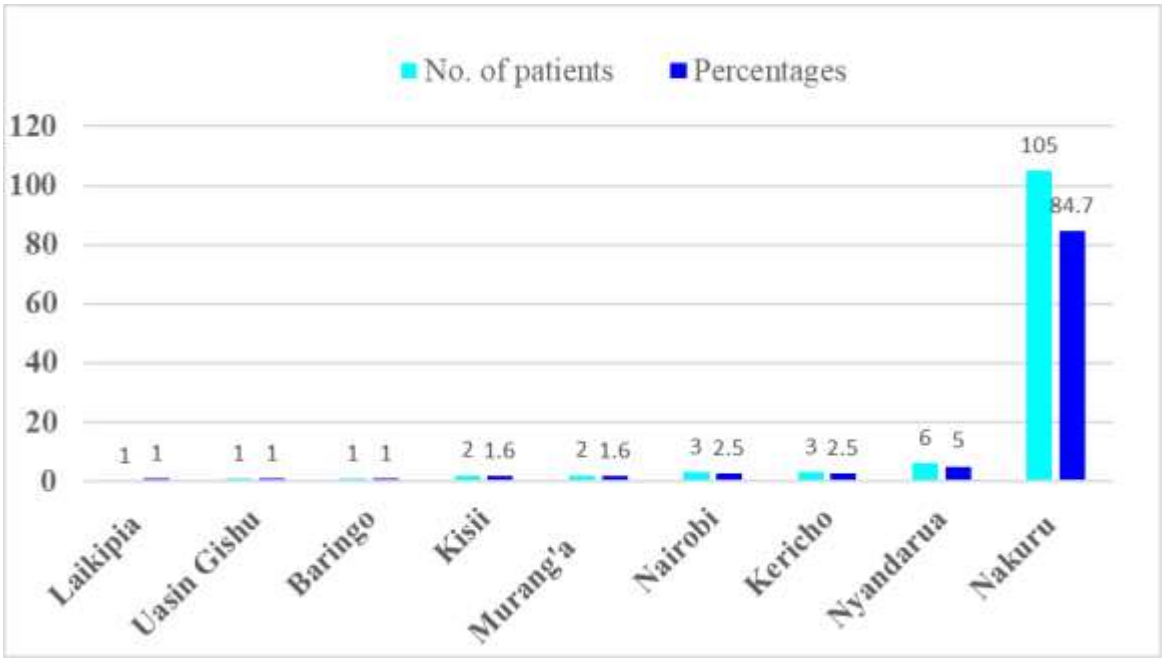


Figure 8: Region of Residence of study participants

IV) Ethnicity

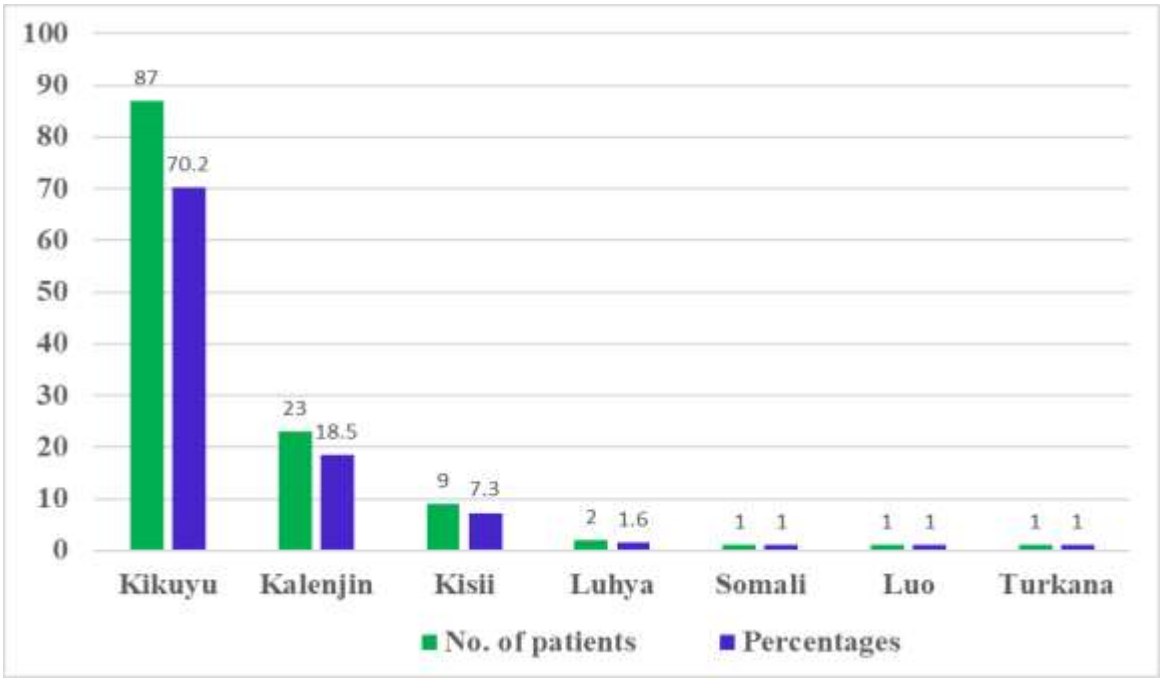


Figure 9: Distribution of patients by ethnicity.

Majority of the patients 87 (70.2%) were from the Kikuyu ethnicity. Others 37 (29.8%), were distributed among the other six ethnic groups (**fig.9**)

V) Level of Education

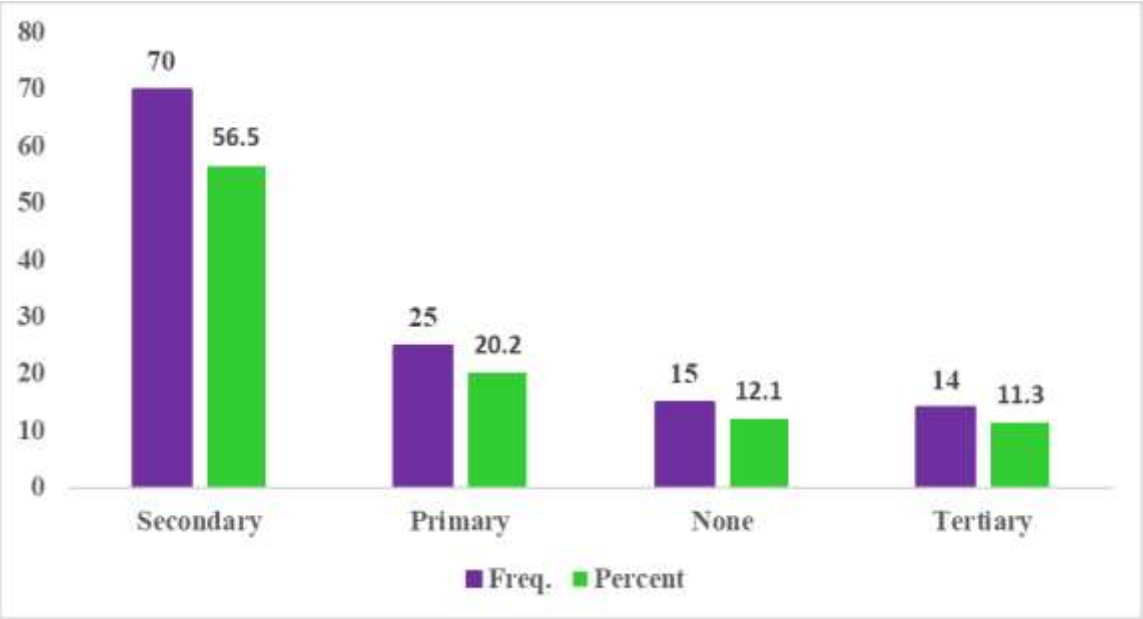


Figure 10: Distribution of patients by level of education

Majority of the participants, 70 (56.5%) had attained secondary level of education, 25 (20.2%) had primary education, 14 (11.3%) tertiary level and 15 (12.1%) never attended any formal education (**fig. 10**).

4.2 Clinical presentation

The most common and major clinical symptoms at presentation were related to goitre, such as anterior neck swelling 95 (76.6%), followed by signs and symptoms associated with hyperthyroidism e.g. Palpitations, sweatings and weight loss at 94 (75.8%), Compression of the trachea or esophagus, such as dyspnea (shortness of breath) on exertion, hoarseness of voice and dysphagia (swallowing difficulties) 84 (67.7%) and Painful neck 62 (50.0%). Fifty (40.0%) of the patients reported more than one symptoms (**Table 1)**

Table 1: Most common presenting symptoms

Symptoms	**Total, n=124, (%)**
Anterior neck swelling (Goitre)	95, (76.6%)
Signs of hyperthyroidism (Sweating, Weight loss & Palpitation)	94, (75.8%)
Compressive Symptoms (Dysphagia, Dyspnea & Hoarseness)	84, (67.7%)
Painful neck	62, (50.0%)

4.3: Characteristics of common thyroid disorders

4.3.1 Biochemical characterization

In biochemical characterization, the following serum biomarkers were used to characterize 'functional' thyroid disorders (abnormal thyroid function) which include:

I. Tetra-iodothyronine / Thyroxine (T_4) and Tri-iodothyronine (T_3)

II. Thyroid stimulating hormone (TSH)

III. Anti-Thyroglobulin antibodies (anti-TgAB) and Anti-Thyroid peroxidase antibodies (anti-TPOAb)

IV. Thyroid stimulating immunoglobulin (TSI)

V. Serum inorganic iodide (I^-) levels

One hundred and twenty patients (**120**) required biochemical assays for thyroid function status using the above biomarkers, 45% 54 patients were diagnosed with 'functional' thyroid disorders (abnormal thyroid function) while the rest were Euthyroid.

And based on these biomarkers, the following 'functional' thyroid disorders were characterized:

Hyperthyroid (Thyrotoxicosis): (TSH level ≤ 0.2 µIU/L, T_4 level > 11.6 µg/dl and Iodide (I^-) level >18 µg/dl). And out of the **48** patients diagnosed with hyperthyroidism (**fig.11**), 54.2% (n=26) patients had nodular goitres (25 multi-nodular goitres & 1 Cystic nodular goitre), 39.6% (n=19) were diagnosed with Graves' disease after thyroid stimulating immunoglobulin (TSI) assays were performed and three (**3**) cases with thyroid carcinoma were also hyperthyroid (**Table 5**).

95.8% (n=46) were females and majority were in the age bracket 21- 60 years which constitutes 79.2% of the hyperthyroid patients. Forty-three (**43**) of the patients, which correspond to 89.6% had overt thyrotoxicosis (raised T_4 and T_3) and all were females (**Table 2).**

Euthyroid: TSH, T_4, T_3 and Iodide (I^-) levels within their normal ranges, (0.2 - 5.2 µIU/L, 4.1 – 11.6 µg/dl, 1.1 – 4.6 ng/ml and 5 – 18 µg/dl) respectively). Out of the **120** patients who were done thyroid hormonal profile, **66** were Euthyroid **(fig. 11).** Among the Euthyroid group, 68.2% (n=45) were found in the age bracket between 21 – 60 years. Among this group **8** patients were diagnosed with simple diffuse goitre and **52** patients had nodular goitre. Also among the Euthyroid group **3** patients had been diagnosed with solitary thyroid nodules (STN) and another **3** patients were found to have thyroid carcinoma (**Table 2 & 5**)

Hypothyroid: (TSH level >5.2 µIU/L, T_4 level <4.1 µg/dl and Iodide (I^-) level <5.0).

Only one (**1**) case of overt hypothyroidism (low T_4 and T_3 levels), female of 35 years who had multi-nodular goitre (endemic goitre) due to Iodine deficiency disorder

(IDD) was diagnosed with hypothyroidism among the study patients (**fig. 11 & table 2**).

Subclinical hyperthyroid: (TSH level, <0.2 µIU/L and normal T_4, T_3 and Iodide (I^-) levels) (4.1 – 11.6 µg/dl), (1.1 – 4.6 ng/ml) and (5.0 – 18 µg/dl) respectively.

Four, (3.3%) of the patients who were diagnosed with subclinical hyperthyroidism had multi-nodular goitre and all were females **(fig.11 & table 2**).

Subclinical hypothyroid: (TSH level >5.2 µIU/L and normal T_4, T_3 and Iodide (I^-) levels) (4.1 – 11.6 µg/dl), (1.1 – 4.6 ng/ml) and (5.0 – 18 µg/dl) respectively.

One (**1**) female participant of 56 years who had multi-nodular goitre was diagnosed with subclinical hypothyroidism **(fig. 11 &Table 2).**

Graves' disease: (Thyrotoxicosis + Thyroid stimulating immunoglobulins level >1.5 IU/L). Normal ranges, 0 – 1.5 IU/L

Out of the total **48** patients with hyperthyroidism, 39.6% (n=19) were diagnosed with Graves' disease and majority, 94.7% (n=18) were females with 63.2% (n=12) were in the age bracket of 21 – 40 years **(Table 4 & 5**).

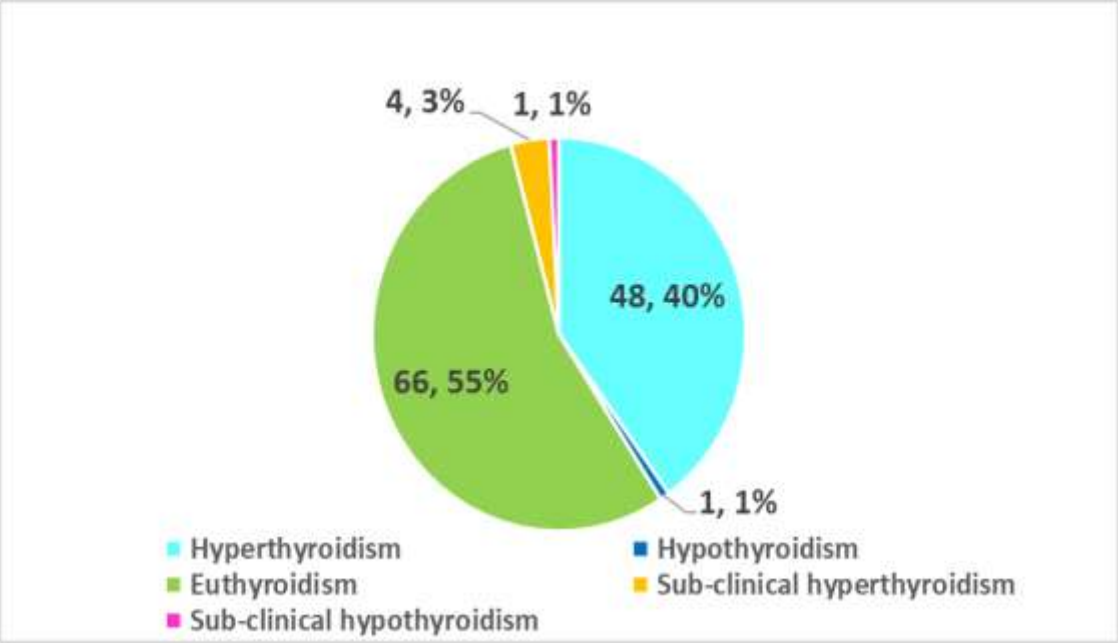


Figure 11: Distribution of patients with 'functional' thyroid disorders based on biochemical characterization.

Table 2: Distribution of patients with different thyroid function status by age and gender

Demographic characteristics		**Thyroid hormonal profile (Thyroid function status)**				
Age in years	Gender	Hyperthyroidism	Hypothyroidism	Euthyroidism	SC hyperthyroidism	SC hypothyroidism
0 - 20	F	7	0	2	1	0
	M	0	0	0	0	0
21 - 40	F	20	1	29	1	0
	M	0	0	2	0	0
41 - 60	F	18	0	16	2	1
	M	2	0	6	0	0
61 - 80	F	1	0	11	0	0
	M	0	0	0	0	0
TOTAL, N=120		48	1	66	4	1
PERCENT (%)		40%	1%	55%	3%	1%

4.3.2 Non biochemical characterization

In non-biochemical characterization, the following investigation techniques were used to characterize 'structural' thyroid disorders which include:

Thyroid ultra sound imaging – For the detection and diagnosis of goitre, thyroid cancer and solitary thyroid nodules, and was performed in **123** of the patients as indicated in the radiological request forms. Thyroid ultra-sound imaging detected **86** cases of nodular goitres, **27** cases of diffuse goitres, **7** cases of thyroid carcinomas and **3** cases of solitary thyroid nodules.

FNA/ Histology – For the determination and confirmation the nature of goitres / cystic nodules in suspected cases of thyroid carcinomas. FNA was performed in **15** patients suspected to have thyroid malignancy (cancer). Out of these, **7** cases were confirmed to have thyroid carcinomas through histological examination.

And based on the above investigation techniques the following 'structural' thyroid disorders were characterized:

Goitres

Goitre was the most common thyroid disorder diagnosed, representing 76.6% (n =95) of all the cases, followed by Graves' disease at 15% (n=19), **(Fig. 13)**. And out of ninety-five patients with goitre, 91.6% (n=87) of them were females with 74.7% (n=71) were in the age category 21 – 60 years. 91.6%, (n=87) of all goitres were nodular compared with simple diffuse 8.4% (n=8) (**Table 4**). Among the patients with goitres, 27.4% (n=**26**) were diagnosed with toxic nodular goitre after thyroid hormonal profiles were assayed, also had signs and symptoms associated with hyperthyroidism. 54.7% (n=**52**) of all nodular goitres were Euthyroid / Simple goitres (**Table 5**).

Goitres can be further classified based on their morphology and growth pattern as follows, toxic multi-nodular, simple / non-toxic multinodular, simple diffuse, cystic, solitary / Uni-nodular, colloid, recurrent MNG and endemic MNG (**Table 3 & Fig. 12**)

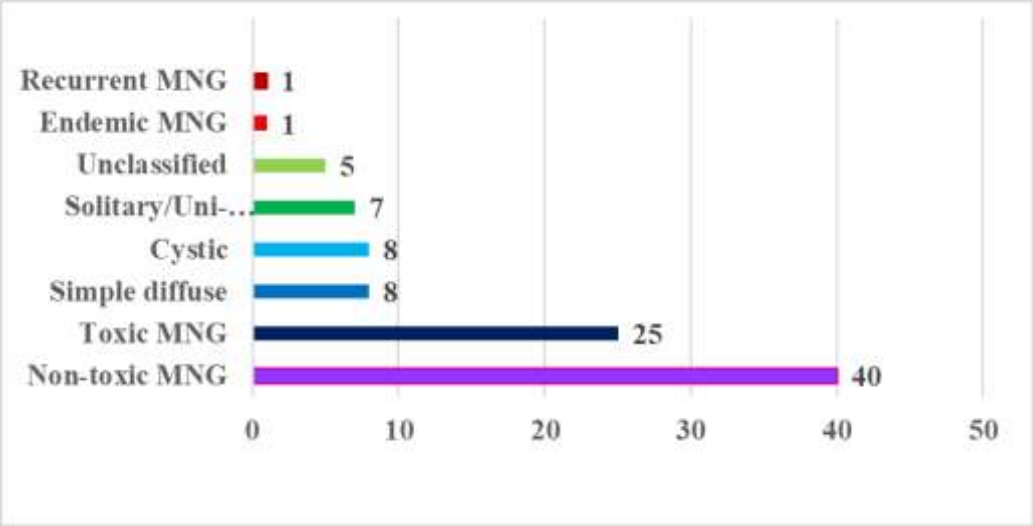


Figure 12: Description of the overall characteristics of goitre types at Nakuru level 5 Hospital

Table 3: Distribution of Goitres types in relation with Gender & Age

	TYPES OF GOITRES								
Age in years	Gender	Toxic-MNG	Simple MNG	Simple diffuse	Cystic	Solitary	Recurrent MNG	Endemic MNG/IDD	Unclassified
0 – 20	F	3	0	0	1	0	0	0	1
	M	0	0	0	0	0	0	0	0
21 – 40	F	10	17	4	2	3	0	1	1
	M	0	0	0	1	1	0	0	0
41 - 60	F	11	14	2	3	0	1	0	3
	M	1	0	2	1	2	0	0	0
61 – 80	F	0	9	0	0	1	0	0	0
	M	0	0	0	0	0	0	0	0
TOTAl, N=95		25	40	8	8	7	1	1	5
PERCENT (%)		(26.3%)	(42.1%)	(8.4%)	(8.4%)	(7.4%)	(1.1%)	(1.1%)	(5.3%)

Solitary Thyroid nodules (STN)

A total of three (**3**) patients with solitary thyroid nodules (2 cystic & 1 colloid) which represent 2.4% of the thyroid cases were diagnosed among the participants in the study **(Fig 13**). All the three (3) cases were Euthyroid and two (**2**) of the participants were females in the age bracket of 21 – 40 years and the only male with colloid nodule was 44 years old (**Table 4**).

Thyroid carcinoma (CA)

Thyroid cancer (malignancy) was suspected in 12.1% (n=15) of the cases and Fine needle aspirate for cytology (FNAC) and biopsy were performed and 46.7% (n = 7) had cytology findings suspicious for thyroid cancer or malignancies and were confirmed through histological examination (**Fig 13**). Six (**6**) of the cases were follicular CA and one (**1**) was anaplastic CA. All the seven (**7**) confirmed thyroid carcinomas were females with their ages ranging between 41 – 80 years (**Table 4**). Three (**3**) of the patients with follicular thyroid carcinoma were also hyperthyroid and the remaining three (**3**) were Euthyroid. (**Table 5**)

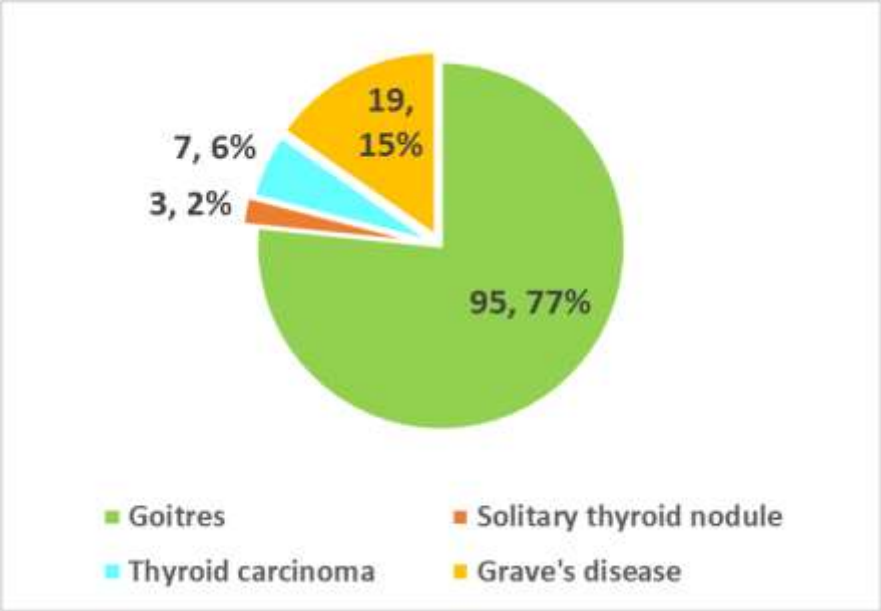


Figure 13: Distribution of patients with thyroid disorders based on non- biochemical characterization.

Table 4: Distribution of patients with "structural" thyroid disorders in relation with Gender and Age

		THYROID DISORDERS							
Age in years	**Gender**	Goitres		Graves' disease	Thyroid carcinomas		STN		N, (%)
		Nodular	Diffuse		Follicular	Anaplastic	Cystic	Colloid	
0 - 20	F	5	1	3	0	0	0	0	9, (7.3%)
	M	0	0	0	0	0	0	0	0, (0%)
21 - 40	F	34	4	12	0	0	1	1	52, (41.9%)
	M	2	0	0	0	0	0	0	2, (1.6%)
41 - 60	F	32	1	3	3	1	0	0	40, (32.3%)
	M	4	2	1	0	0	1	0	8, (6.5%)
61 - 80	F	10	0	0	3	0	0	0	13, (10.5%)
	M	0	0	0	0	0	0	0	0, (0%)
TOTAL		87	8	19	6	1	2	1	124
PERCENT (%)		70.2%	6.5%	15.3%	4.8%	0.8%	1.6%	0.8%	100%

Table 5: Comparison of thyroid disorders with thyroid function status

	THYROID DISORDERS					
Thyroid function status	Goitres		Graves' disease	Thyroid carcinoma	STN	Total, (%)
	Diffuse	Nodular				
Hyperthyroidism	0	26	19	3	0	48, (40%)
Hypothyroidism	0	1	0	0	0	1, (0.8%)
Euthyroidism	8	52	0	3	3	66, (55%)
Sub-clinical hyperthyroidism	0	4	0	0	0	4, (3.3%)
Sub-clinical hypothyroidism	0	1	0	0	0	1, (0.8%)
TOTAL, N=120	8	84	19	6	3	120, (100%)
PERCENT (%)	6.7%	70.0%	15.8%	5.0%	2.5%	100%

Clinical presentation / characteristics of the study patients

Painful neck was a common findings in patients with goitre. It was found in 50.0% of the patients and most of them were goitre cases at 27.4% (**table 6**). Compressive symptoms like hoarseness and dyspnea were common among patients with goitre. Among all patients evaluated, dysphagia and hoarseness were present in 27.4% and 23.4% respectively, while only 16.9% had dyspnea (**table 6**). Palpitations were a frequent symptoms among hyperthyroid patients and Graves' disease. Weight loss was much more common among patients with hyperthyroidism and Graves' disease at 13.7 % and 9.7% respectively (**table 6**). Sweatings which was present in 21.0% of all

the patients seen, was more associated with hyperthyroidism and Graves' disease. Sweatings seem to be related to heat intolerance.

Table 6: Clinical characteristics / features of patients with thyroid disease and their association with thyroid disorders (functional and structural)

Clinical features	Hyperthyroid	Hypothyroid	Euthyroid	SC hyperthyroid	SC hypothyroid	Goitre	Graves' disease	Thyroid CA	STN	Total=124 n, (%)
Painful neck	6	1	10	1	-	34	7	2	1	62, (50.0)
Dyspnea	5	-	3	-	-	12	-	1	-	21, (16.9)
Dysphagia	9	-	8	-	-	14	-	3	-	34, (27.4)
Hoarseness	6	-	8	-	-	15	-	-	-	29, (23.4)
Palpitation	24	-	1	-	-	-	14	-	-	39, (31.5)
Weight loss	17	-	-	-	-	-	12	-	-	29, (23.4)
Sweatings	12	-	-	-	-	-	14	-	-	26, (21.0)
Tremors	6	--	-	-	-	-	8	-	-	14, (11.3)
HTN / DM	-	-	3	-	-	-	-	-	-	3, (2.4)
Headache	2	-	-	-	-	-	-	-	-	2, (1.6)
Exophthalmos		-	-	-	-	-	4	-	-	4, (3.2)

4.4 Types and proportions of thyroid disorders at Nakuru level 5 Hospital

The distribution of specific thyroid disorders seen in the study population of patients at Nakuru level 5 Hospital surgical clinic were as follows:

Table 7: Types and proportions of 'functional' thyroid disorders at Nakuru level 5 Hospital

'**Functional' Thyroid disorders**	**Total n=120, (%)**
Euthyroidism	66, (55%)
Hyperthyroidism	48, (40%)
Sub-clinical hyperthyroidism	4, (3%)
Hypothyroidism	1, (1%)
Sub-clinical hypothyroidism	1, (1%)

Table 8: Types and proportions of 'structural' thyroid disorders at Nakuru level 5 Hospital

'Structural' Thyroid disorders	**Total n=124, (%)**
Goitres (all)	95, (77%)
Graves' disease	19, (15%)
Thyroid carcinoma	7, (6%)
Solitary Thyroid nodule (STN)	3, (2%)

CHAPTER FIVE

5.0 DISCUSSION

The study was a descriptive cross-sectional study of patients with thyroid disorders or signs and symptoms of thyroid disorders who were referred and presented themselves to Nakuru level 5 Hospital, surgical clinic. It documented the socio-demographic characteristics, clinical presentation, serum thyroid hormonal profile, thyroid auto-antibodies assays, serum iodide levels estimation, thyroid ultrasound imaging and Fine needle aspirate for cytology, for the overall assessment of thyroid function status of the patients. This study was carried out at Nakuru level 5 Hospital because it is the largest regional Referral health facility in South and Central Rift Valley and cases of thyroid dysfunctions both from within the surrounding communities and neighbouring counties (Bomet, Baringo, Narok, Kericho, Samburu, Laikipia and Nyandarua) are handled at the tertiary healthcare facility. Other studies have been carried out at Kijabe mission Hospital on retrospective review of all thyroidectomies over a three-year period from 1st January, 1999 to 31st December, 2002 (Hill et al. 2004). None of the studies had combined use of thyroid hormonal profile, serum iodide levels and anti-thyroid antibodies assays to assess thyroid function as a reference method of assessing thyroid dysfunction. Thyroid disease at Nakuru level 5 Hospital is predominantly a female condition with the majority of the patients in this study being females at 91.9%, giving male to female ratio of 1:11. This is in sharp contrast to Mwangi and Hills findings of male to female ratio of 1:7 (Hill et al. 2004). The male to female ratio in this study is almost relatively consistent with findings in another study of thyroid disease among patients attending New Mulago Hospital thyroid clinic (Mutakirwa et al. 2001).

Thyroid disorders clearly affect more women than men owing to the presence of estrogen receptors in thyroid tissue and vary according to gender, genetics and environmental factors. There is also a congenital predisposition as well. Women are less susceptible to infection diseases than men but are more often prone to autoimmune diseases. This higher prevalence is attributed to the X-chromosomes which has many genes relating to the immune system in addition to the differences in sex hormones i.e. estrogen which modulate autoimmune diseases function and favours the antibody production-enhancing Th2 response and, possibly, enhances towards abnormal autoimmune functional risk, could explain the increased frequency of thyroid dysfunction in females than males and also altered metabolic status which a female has to go through namely, menstruation, pregnancy and lactation (Fairweather et al. 2008)

Eighty-two per cent, (n=102), of thyroid disorders of which females constitute 90.2% were found in the age group 21– 60 years while the extremes of age, below 20 years and over 60 years had the least (7.3% and 10.5%, respectively) with no male representative. This is almost similar to the findings in retrospective analysis of patients' biopsies with thyroid disease at Tikur Anbessa teaching and referral hospital, Addis Ababa University. This confirms that thyroid diseases are not common in the extremes of age (Tsegaye et al. 2003).

Sixty-four percent (n=79) of the patients were in the age category of 21 – 50 years and 89.9% (n=71) were females. This corresponds to the peak age incidence commonly affected by auto-immune thyroid disease (Faggiano et al. 2011).

Most patients 84.7% were from Nakuru County. This is expected since Nakuru level 5 Hospital is located in Nakuru County, thus conveniently accessible by the local

populace. Also, the health facility is the largest regional referral Hospital in central and south Rift Valley, Kenya

There was a strong tribal biased in presentation to the hospital, Kikuyu was the predominant tribe represented at 70.2%, reflecting the local area population. This is similar to the findings by Mwangi and Hills in which Kikuyu representation was 61% (Hill et al. 2004). Other tribes were represented as follows, Kalenjin 18.5%, Kisii 7.3%, Luhya 1.6%, Somali 0.8%, Luo 0.8% and Turkana 0.8%. Further studies need to be carried to assess cases of thyroid disorders among these minority tribes in their regions of residence where they are the majority to find out if the findings reflect the same.

Majority of the patients, 68% (n=84) had attained at least secondary level of education, this reflects higher level of awareness and knowledge of understanding the importance of visiting health facilities for medical care.

The major and most common clinical presentation among the study participants was anterior neck swelling (goitre) at 76.6% and this is in consistent with similar findings by Abebe and Mensur on prospective study of patients with goitres between December, 2003 to August, 2004 in Ethiopia (Bekele et al. 2006). Signs and symptoms of hyperthyroidism e.g. Palpitation, sweating and weight loss follow at 75.8%. Compressive symptoms e.g. dyspnea, dysphagia and hoarseness were present in 67.7% of the study participants. This is in sharp contrast to CA Banks findings of 52% on retrospective review of patients who underwent thyroidectomy between 2005 – 2009 (Banks et al. 2012). Palpitations were present in 50.0% of all the patients with hyperthyroidism and there was a significant association between palpitation and hyperthyroidism. Sweating and weight loss were also associated with hyperthyroidism. The presence of these symptoms were comparable to that found

among eighty-four thyrotoxic patients with overt hyperthyroidism in a prospective cohort study (Trivalle et al. 1996).

Out of 120 patients who had undergone biochemical characterization, 92 of them had earlier been diagnosed and characterized to have goitre. Among this group with goitre, 84 patients had nodular goitre and 8 had diffuse goitre. 21.7% (n=26) of the patients with nodular goitre were diagnosed / characterized as hyperthyroid with toxic goitres after performing thyroid hormonal assays (25 multi-nodular goitres & 1 cystic nodular goitre). Of the 48 patients who were characterized biochemically, as hyperthyroid, Thyroid stimulating immunoglobulin assays (TSI) were assayed and 19 of these patients were diagnosed with Graves' disease. All the 8 patients diagnosed with diffuse goitre were characterized as Euthyroid / simple diffuse goitre after thyroid function status were analyzed. Four (4) patients with nodular goitre were characterized as having subclinical hyperthyroidism and also one (1) patient had subclinical hypothyroidism. Also among the patients with nodular goitre, one (1) case was diagnosed with overt hypothyroidism, whose serum was further analyzed for the presence of anti-Thyroid peroxidase antibodies (anti-TPO abs) and anti-Thyroglobulin antibodies (anti-Hg abs) assays and the laboratory results ruled out Hashimoto's disease / thyroiditis. 52 cases of nodular goitre were diagnosed / characterized biochemically as Euthyroid / simple nodular goitre.

Thyroid function status was investigated on the basis of clinical signs and symptoms of thyroid dysfunction of the study patients. Serum thyroid hormonal profile were assayed and measurements of anti-thyroid antibodies levels as an additional assays. Forty-three patients (43) out of forty-eight (48) patients, representing (89.6%) presented with overt thyrotoxicosis and all were females, four (4) patients with subclinical hyperthyroidism had low serum TSH and normal T_4, T_3 and iodide levels

Similarly, one patient with overt hypothyroidism and one (1) with subclinical hypothyroidism had both high serum TSH levels, low T_4, T_3, iodide levels and normal T_4, T_3, iodide levels respectively. TSH levels are normally assayed as the first line of testing (screening test) in determining thyroid status, whether normal (Euthyroid), hyper functioning (hyperthyroid), or hypo functioning (hypothyroid). This is in agreement with Ross findings in which he shows that TSH level was valuable in differentiating hyperthyroid and hypothyroid patients from Euthyroid patients (Ross et al. 2001). Five (5) patients with features of hyperthyroidism had normal serum T_4 but elevated serum T_3 levels (T_3 thyrotoxicosis) and their common pathological cause was multinodular goitre. The majority of patients suffering from T_3-thyrotoxicosis have autonomous thyroid function (i.e. toxic Uninodular and multinodular goitres) and this form occurred in 11% of untreated thyrotoxicosis. Tri-iodothyronine (T_3) is a sensitive marker of endogenous hyperthyroidism and in levothyroxine (T_4)-induced hyperthyroidism, there is no reason for T_3 to be elevated (Konrady et al. 2000). In this study patients diagnosed for hyperthyroidism, diffuse toxic goitre with or without opthalmopathy were further tested for thyroid stimulating immunoglobulin (TSI) to confirm or rule out Graves' disease. Graves 'disease was diagnosed in 39.6 % (n=19) of all patients with hyperthyroidism and this represents 15.3% of all the study patients. This is nearly comparable with Mwangi and Hills findings in retrospective review of thyroidectomies at a rural church Hospital in Kenya (Hill et al. 2004). Of the 120 patients done serum iodide levels, 48 patients showed slight raised iodide levels >18 µg / dl, while one (1) case with hypothyroidism had low iodide levels < 5.0 µg / dl. All other cases who were Euthyroid had serum iodide levels within their normal ranges. Thyroid ultra-sound imaging detected 95 cases of goitres, among the referred cases and out of these. 87 cases were nodular and 8 cases were characterized

as diffuse goitres. Thyroid ultra- sound imaging further classified nodular goitre as follows: multiple nodules, solitary / Uni-nodular, cystic and colloid goitres. Also thyroid ultra-sound imaging detected 3 cases of solitary thyroid nodule (STN) which were classified as two (2) cystic and one (1) colloid / solid nodules.

Utilization of thyroid ultrasound imaging was greater than thyroid function tests at 99.2% and 96.8% respectively. Thyroid ultrasound imaging detected accurately more goitres than on clinical examination, this signifies the importance of conventional ultrasound imaging in classifying goitres as nodular or diffuse thyroid disorders, solid / colloid, cystic or mixed with precise accuracy. This helps in therapeutic aspiration of cystic nodules and diagnostic aspiration for cytology of multiple nodules to rule out malignancy (Nyonyintono et al. 2011).

Also out of 124 patients referred and presented themselves at surgical clinic, 15 patients required Fine needle aspirate for cytology (FNAC) investigations as indicated and 7 of them, all females had cytological findings suspicious of malignancy and were confirmed through histopathological technique (6 follicular CA & 1 anaplastic CA). The number of patients aspirated in the study was very small due to limited span of the study period and few cytological requests.

The most common thyroid disorder diagnosed in this study was goitre 76.6% (n =95) with most patients presented with complaints of anterior neck swelling. Goitre continues to be an endemic problem in many parts of the world (Carle et al. 2014). Clinically, and ultrasound imaging, goitres varied from diffuse goitres, 8.4% (n=8) to nodular goitres, 91.6% (n=87). Prevalence of goitres among the patients studied was consistent with similar findings in endemic and non-endemic areas in sub-Saharan Africa (Sidibe el et al. 2007).

This study also raises several clinical questions, hyperthyroidism was diagnosed in 40% (n=48) of patients done thyroid hormonal profile, which is high for a region in Rift valley, Kenya which is known for endemic goitre due to iodine deficiency and simple goitres. The etiologies for this finding were nodular goitres, 54.2%, (25 MNG & 1 cystic nodular goitre) and Graves' disease 39.6%, (n=19). These results may reflect the conversion of non-functioning nodules to hyper functioning state after iodine supplementation (Jod-Basedow effect - hyperthyroidism following administration of iodide), and could represent the transition from iodine deficient to iodine sufficient states. Other possibilities could include autonomous hyper functioning nodules to compensate for hypothyroidism. Subclinical and clinical hypothyroidism represent 1.6% (n=2) while subclinical hyperthyroidism cases were 3.2% (n=4).

Thyroid cancers were not uncommon findings at 5.6%, (n=7) with follicular carcinoma constituting 85.7% (n=6) cases which is similar to the findings by other authors (Chalya et al. 2011; Solomon et al. 2015), while anaplastic carcinoma represents 14.3% (n=1). Solitary thyroid nodule (STN) were rare at 2%, (n=3) and none was malignant.

5.1 Limitations of the study

This study centered only on those patients who were symptomatic due to advanced disease state and thus willing to obtain surgical intervention as a last resort. Therefore, the population studied in this case may not be fully representative of the general population of patients with thyroid dysfunctions.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSIONS

I. The age bracket commonly affected by thyroid disorders was 20 – 50 years, which represents 63.7%.

II. The commonest clinical presentations was anterior neck swelling (goitre) at 76.6%,

III. Functional thyroid disorders can be successfully characterized using biochemical markers whereas structural thyroid disorders by using non-biochemical techniques.

IV. Goitre accounts for the highest (77%) of all thyroid patients, and solitary thyroid nodules the least at 2.0%.

V. The commonest cause of hyperthyroidism among the hyperthyroid patients was toxic nodular goitre at 54.2% and is most common in females. Therefore gender is a risk factor of hyperthyroidism since prevalence is common in females at 54.2%.

6.2 RECOMMENDATIONS

I. Further studies are required to elucidate the risk factors contributing to the increase cases of goitres especially among females in the Central Rift Valley region of Kenya. The target age group (21- 50 years).

II. Prospective studies need to be undertaken to further determine the possible etiological factors to why there is a changing trend in thyroid carcinoma from the more common papillary cancer to a rare follicular cancer.

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APPENDICES

Appendix I: Questionnaire

Questionnaire for clinical profile and thyroid function (Investigations) in patients attending Surgical Clinic at Nakuru level 5 Hospital

Study Title: Characterization of Thyroid disorders among Patients attending surgical clinic at Nakuru level 5 Hospital.

Patient Name:_____

Birth Date_____

Patient Number:_____

Date (Interview)_____

Gender _____ Marital Status_____ Age _____ Ethnicity:_____

Income Source_____

Education: None ☐ Primary ☐ Secondary ☐ Tertiary ☐

Birthplace (District)_____Religion:_____ Residence_____

Chief Complaint: Swelling /Mass in ne☐ Dysphasia☐ Pain/ Tenderness ☐ in neck

Other (specify) _____

Duration of acute / presenting symptoms:

Clinical presentation: ☐ressure ☐ Toxicity☐ Malignanc☐ Cosm☐c Others

Specify all symptoms:_____

Duration of Symptoms: _____

_____ ☐

Risk Factors: Hx of endocrine disease? ☐ No ☐ DM Thyroid ☐ Other _____

Familial endocrine disease? ☐ No ☐ DM ☐ Thyroid ☐ other_____

Hx of Cancer: No ☐ Breast ☐ Ovarian ☐ Other._____

Hx of treatment with radiation to neck or head? Yes ☐ No Specify:_____

Hx of traditional therapy? Yes ☐ No ☐ Specify: _____

Number of children: (deliveries): Male_____Female_____

Dietary intake: Cassava_____Cabbage_____Soya Beans_____Other (Specify).

PMHx: (RAI and other treatment, specify)

Medications: Allergies:

Thyroid Function Tests

1. Date_____ TSH…….µIU/L T_4: ……..µg/dl T_3…….. ng/ml

2. Date……Anti-TPO……IU/ml

3. Date……Anti-Tg……IU/ml

4: Date……..TSI……IU/L

5: Date……Serum Inorganic Iodine …….µg/100mls

Imaging: U/S: ☐ FNA: ☐

Thoracic Inlet: ☐

X-ray ☐ IDL: ☐ Normal ☐ Abnormal ☐

Physical Exam: Thyroid (lobes involved) ☐ Lymph nodes (specify) ☐ ☐

others

Diagnosis:

Treatment plan:

APPENDIX II: Consent to participate in a research study.

CODE OF THE PARTICIPANT_____

TITLE: CHARACTERIZATION OF THYROID DISORDERS AMONG PATIENTS ATTENDING SURGICAL CLINIC AT NAKURU LEVEL 5 HOSPITAL

My name is Mr. David Ruto, Principal Investigator from the department of Medical Biochemistry (Moi University). I am conducting a research study on characterization of thyroid disorders among patients with thyroid disease. The study will help in the availability of data on biochemical and non-biochemical characterization of common thyroid disorders which is the key to better and effective management of patients with thyroid disease.

Study procedures

If you agree to take part in this research study, the interviewer will fill a form that will capture your personal details like your age, marital status, and education level, socio-economic and socio-demographic characteristics. You will be asked further questions to find out if you have been consuming certain food classes' e.g. Cassava, cabbage, soya beans etc.

5.0 mls of blood will be collected from you. This procedure may cause slight discomfort. Samples will be analyzed at Nakuru level 5 Hospital, clinical laboratory. Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you decline to participate you will not be denied any services from this Health Institution.

Confidentiality:

All the information obtained will be strictly confidential and data password protected and only accessed by the Principal investigator, participants in the study will be kept anonymous, being identified only by specific unique numbers (codes) assigned by the principal investigator.

Benefits:

By choosing to participate in this study, you will have direct benefits by having your disease status re-assess and characterized and laboratory, cytological and radiological examination done at no cost. These expenses will be catered by the principal investigator. This will also aid the clinicians to treat your ailment accordingly.

Risks

There is discomfort and pain in obtaining the blood samples, there are no other foreseeable risks that will arise from participating in the study. You will however take about thirty minutes longer to go through the study procedures.

Written consent:

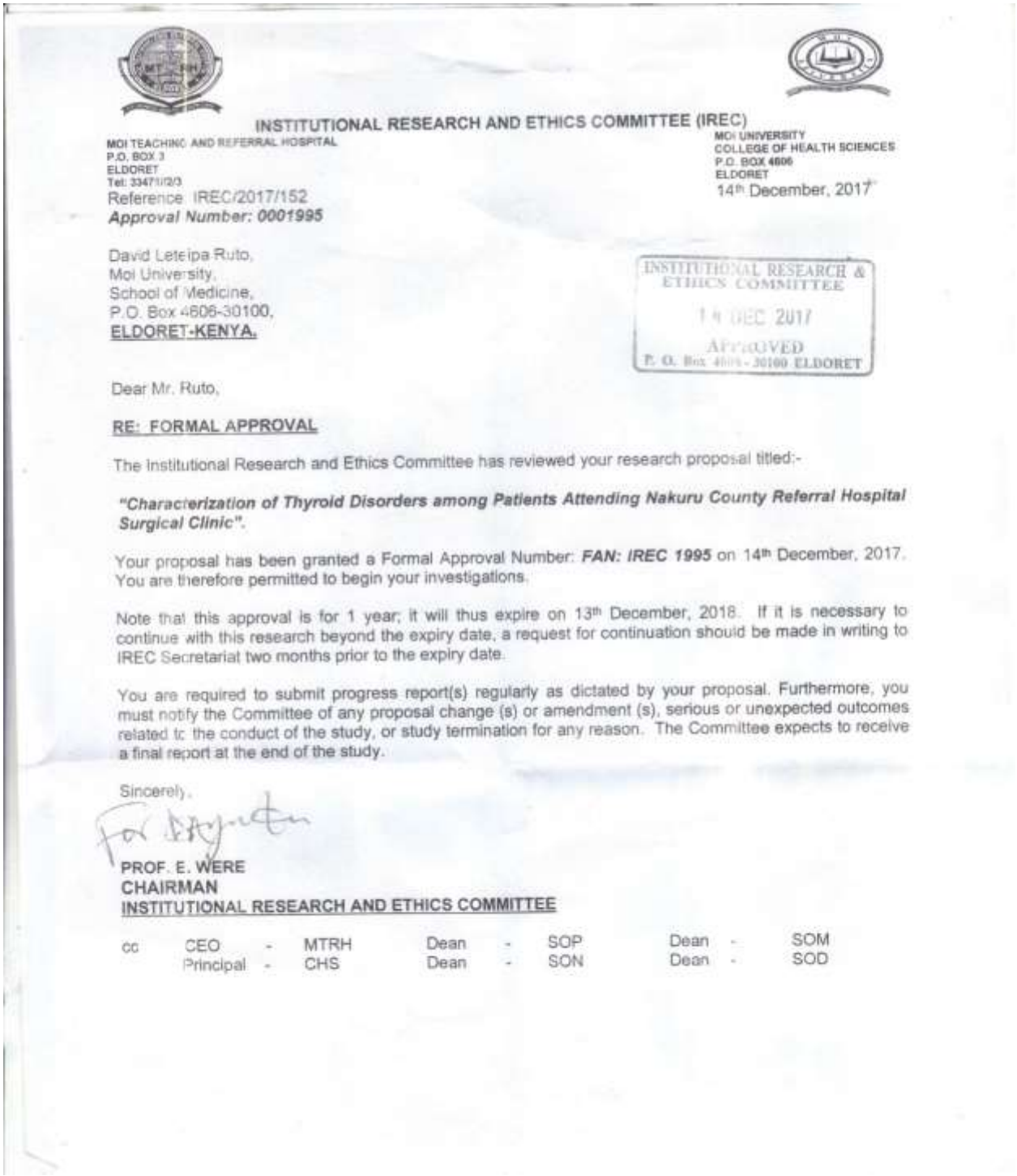
I, the undersigned have understood the above information which has been fully explained to me by the principal investigator. I have agreed to voluntarily consent to participate. I was given the chance to ask questions and I received satisfactory responses.

Signature of participant …… Date …….

I certify that I have followed the study SOP to obtain consent from the participant. She/he has understood the nature and the purpose of the study and consent to their participation in the study. She/he has been given opportunity to ask questions which have been answered satisfactorily.

Signature of Principal Investigator …… Date …….

Appendix III: Institutional Research and Ethics Committee (IREC) Approval Form



INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

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Reference: IREC/2017/152
Approval Number: 0001995

14th December, 2017

David Leteipa Ruto,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
14 DEC 2017
APPROVED
P. O. Box 4606 - 30100 ELDORET

Dear Mr. Ruto,

RE: FORMAL APPROVAL

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

"Characterization of Thyroid Disorders among Patients Attending Nakuru County Referral Hospital Surgical Clinic".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1995*** on 14th December, 2017. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 13th December, 2018. 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.

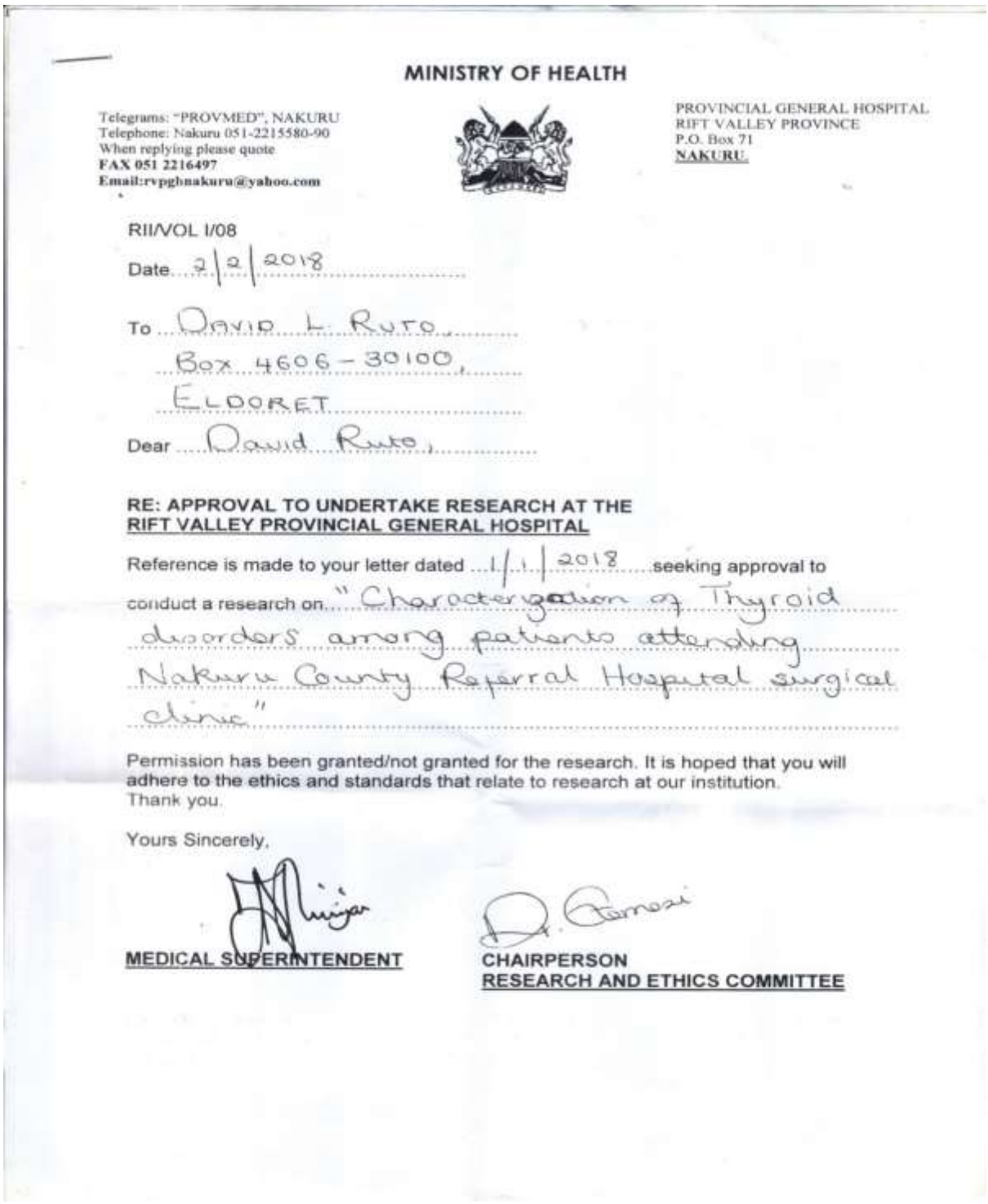
You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects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

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

Appendix IV: Research and Ethics Committee Approval Form To Undertake Research At Nakuru Level 5 Hospital



MINISTRY OF HEALTH

Telegrams: "PROVMED", NAKURU
Telephone: Nakuru 051-2215580-90
When replying please quote
FAX 051 2216497
Email:rvpghnakuru@yahoo.com

PROVINCIAL GENERAL HOSPITAL
RIFT VALLEY PROVINCE
P.O. Box 71
NAKURU

RII/VOL I/08

Date 2/2/2018

To David L. Ruto
Box 4606 - 30100,
ELDORET

Dear David Ruto,

RE: APPROVAL TO UNDERTAKE RESEARCH AT THE RIFT VALLEY PROVINCIAL GENERAL HOSPITAL

Reference is made to your letter dated 1/1/2018 seeking approval to conduct a research on "Characterization of Thyroid disorders among patients attending Nakuru County Referral Hospital surgical clinic"

Permission has been granted/not granted for the research. It is hoped that you will adhere to the ethics and standards that relate to research at our institution.
Thank you.

Yours Sincerely,

MEDICAL SUPERINTENDENT

CHAIRPERSON
RESEARCH AND ETHICS COMMITTEE

Appendix V: Procedure for Serum inorganic iodide determination (modified, S. K. Giri et al.2007)

Chemicals and reagents used

Potassium Dichromate crystals

Sodium carbonate crystals

0.001M Sodium Thiosulphate (standardized)

Tungstic acid (1 part 2/3N Sulphuric acid + 1 part 10% Sodium tungstate)

Distilled Water

1.0 M Hydrochloric Acid

1% Starch

Glass apparatus

50 ml Volumetric Flask

50mls Conical Flasks

Watch Glass

Centrifuge tubes (glass)

50mls Measuring Cylinder

50mls Burette and Stand

Pipette

Beaker

Dropper

Spatula

Equipments

Electronic Balance

Hot Plate

Procedure:

1. To 8.0 mls of distilled water in a clean centrifuge tube labelled to correspond to the patient's code, 1.0 mls of serum was added and mix.
2. 0.5 mls of 2/3N Sulphuric acid was added, followed by 0.5 mls of 10% sodium tungstate
3. The mixture was then thoroughly mix and centrifuged at 3000 rpm for 5 minutes.
4. The whole supernatant poured into the 50mls conical flask containing 0.01gm of potassium Dichromate and 0.2gms of sodium carbonate. The flask was swirled to mix the contents. 1.0 mls of 1M HCI was then added to the conical flask. The flask was then covered with a watch glass and the contents gently mixed.
5. The flask was kept in the dark for 15 minutes. After the specified time, the flask was taken out of the dark and gently swirled.
6. The next step was to place the Sodium Thiosulphate in a 50mls burette, and set it up on a stand using a clamp.
7. The iodine liberated in the flask was then titrated with standardized Sodium thiosulphate. As the titration proceeded, the colour of the solution changes from yellow brown to pale yellow colour, then two drops of starch solution (1%) was added to the mixture in the flask. The starch imparted a blue black colour to the solution since there was the presence of iodine.
8. At the end point of the titration, the solution in the conical flask turns colourless as all the iodine liberated had reacted with the thiosulfate.

The process was repeated three times and the average value for the volume of $Na_2S_2O_3$ determined.

Calculation

Volume of Sodium Thiosulphate used in the titration =V

Concentration of Sodium Thiosulphate = 0.001 M

Number of moles of Sodium Thiosulphate reacting in this titration = $\frac{(0.001 \times V)}{1000}$

Volume of serum used = 1 mIs.

In the above equation, 2 moles of Sodium thiosulphate reacts with 1 mole of Iodine.

Number of moles of Iodine in 1 mls of Serum = $\frac{1}{2} \times \frac{(0.001 \times V)}{1000}$

Number of moles of Iodine in 100 mls of serum = $\frac{1}{2} \times \frac{100}{1} \times \frac{(0.001 \times V)}{1000}$

Concentration of Iodine in grams= $\frac{1}{2} \times \frac{100}{1} \times \frac{(0.001xV)}{1000} \times 127g$ per 100mls of serum

Concentration of Iodine in micrograms (µg) / dl = $\frac{1}{2} \times \frac{100}{1} \times \frac{(0.001 \times V)}{1000} \times 127 \times 10^6$