PREVALENCE AND FACTORS ASSOCIATED WITH IMMUNOLOGICAL THYROID DISEASE AMONG THYROID DISORDER PATIENTS AT MOI TEACHING AND REFERRAL HOSPITAL (MTRH), WESTERN KENYA

BY

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ABSTRACT

Autoimmune thyroiditis is an inflammatory or antireceptor autoimmune condition characterized by reactivity to expressed self-thyroid antigens, and is one of the principal thyroid gland diseases. In Kenya, where the mode of treatment to thyroid disorders is mainly thyroidectomy, documentation of case patterns, prevalence and etiology is still very poor. Therefore this study was done to determine the prevalence, histological patterns, and the levels of thyroid hormones and autoantibodies in immunological thyroid disease patients at Moi Teaching and Referral Hospital (MTRH) in western Kenya. This was a retrospective study in which, samples and data from 388 patients who presented with thyroid pathologies between 2008 and 2011, had thyroidectomy done, and samples taken for histological analysis and diagnosis. Clinical data on thyroid hormones and histopathological diagnosis were extracted from the patient's medical records. Data was analyzed using STATA version SE/10 (College Station, Texas, USA). The results showed that the prevalence levels were; thyroiditis 24 (6.2%), thyroid carcinoma 18 (4.6%), thyroid adenoma 51 (13.1%), colloid goitre 286 (73.7%), thyroid cysts 8 (2.1%) and thyroid abscess 1 (0.3%). Immunological thyroid disease was present in 175 (45%, 95% CL: 40-50) subjects. The median thyroid stimulating hormone levels was TSH 1.8 (IQR: 0.9-2.9), T_3 1.8 (IQR: 1.3-2.7) and T_4 1.2 (IQR: 0.7-1.9). Thyroid stimulating hormone (TSH) and triiodothyronine hormone (T_3) levels for immunological thyroid disease patients were higher (p=0.0232; 0.040, respectively), for those aged 30-39 years. Similarly, creatinine level for immunological thyroid disease patients, median: 58 (IQR: 50-67), were higher (p=0.039) for immunological thyroid disease patients. The presence of the thyroid auto antibodies was significantly associated with the autoimmune thyroid disease (p=0.001). The results showed that the prevalence of immunological thyroid disease was higher (7.5%) than the earlier reported prevalence (2%) by other studies in Kenya and that thyroid hormone levels (TSH) and triiodothyronine hormones (T_3) significantly contribute to the occurrence of immunological thyroid disease. This study has shown that the hormonal, auto antibodies, biochemical and hematological profiles are altered in immunological thyroid disease patients and hence these parameters can serve as a tool for diagnosing thyroid autoimmunity.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Autoimmune thyroid diseases have been reported in people living in different parts of the world including North America, Europe, Baalkans, Asia, Middle East, South America and Africa though the reported figures however do not fully reflect the number of people infected per year (Cureresearch, 2005). Cases are unrecognized due to inaccurate diagnosis and hence are treated as other diseases. In the USA and Canada the extrapolated prevalence is 5,873,108 and 650,157, respectively. In Austria and Belgium, the prevalence is 163,495 and 206,965, respectively. In Bosnia and Macedonia, the prevalence is 8,152 and 40,801, respectively. For China and India, the prevalence is 25,976,952 and 21,301,412, respectively, while in Egypt and Iran it is 1,522,348 and 1,350,064, respectively. South Africa has a prevalence of 888,969 (Cureresearch, 2005) (see appendix x). The annual incidence of Hashimoto's thyroiditis worldwide is estimated to be 0.3-1.5 cases per 1000 persons (Vanderpump *et al.*, 1995) whereas Graves' disease is estimated at about 5 per 10,000 people (Rose, 2008). However, the most recent studies have shown that the human autoimmune thyroid diseases (AITDs) affect up to 5% of the general population (Canaris *et al.*, 2000; Tomer and Huber, 2009).

The principal diseases of the human thyroid gland are goiter (diffuse or nodular), hyperthyroidism, hypothyroidism, autoimmune thyroiditis and neoplasm (Larry *et al.*, 2001). The thyroiditis types cause inflammation of thyroid tissue and can release preformed hormone from the colloid space, causing thyrotoxicosis, which is transient and followed by recovery or development of hypothyroidism (Benbassat *et al.*, 2000; Gharib *et al.*, 2005). In acute and sub acute thyroiditis, thyroid tenderness and neck pain are often present. On the other hand, silent thyroiditis is devoid of the local symptoms (Dorairajan *et al.*, 2002).

The human autoimmune thyroid disorders (AITDs) broadly includes Graves' disease (GD) and Hashimoto's thyroiditis (HT) which are the most common causes of thyroid gland dysfunctions and non endemic goiter (Vanderpump *et al.*, 1995; Larry *et al.,* 2001). These conditions arise due to complex interactions between environmental and genetic factors (Huber *et al.*, 2008; Tomer and Huber, 2009; Hadj-Kacem *et al.*, 2009) and are characterized by reactivity to self-thyroid antigens which are expressed as distinctive inflammatory or antireceptor autoimmune diseases (Weetman and McGregor, 1994; Eguchi *et al.*, 1995; Vanderpump *et al.*, 1995). Among the major AITD susceptibility genes that have been identified and characterized is the HLA-DR gene locus, as well as non-MHC genes including the CTLA-4, CD40, PTPN22, thyroglobulin, and TSH receptor genes (Huber *et al.*, 2008; Tomer and Huber, 2009; Hadj-Kacem *et al.*, 2009). The major environmental triggers of AITD include iodine, medications, infection, smoking, stress and genetic predisposition to AITD which lead to novel putative mechanisms by which the genetic-environmental interactions may lead to the development of thyroid autoimmunity (Tomer and Huber, 2009).

The first pathological features of autoimmune thyroiditis were described in 1912 (Hashimoto, 1912) when patients with goiter exhibited diffuse lymphocyte infiltration, atrophy of follicular cells, presence of granulated thyrocytes (onlocytic cells or Hurtle's cells) and fibrosis in the histological pictures of their thyroid tissues (Hashimoto, 1912). The Hashimoto's thyroiditis disorder is directed against thyroid antigens and is the most common cause of hypothyroidism. The incidence is 0.3 to 1.5 per 1000 persons per year, and it is 4 to 10 times more common in women than in men (Canaris *et al.,* 2000; Huber *et al.*, 2008). Hashimoto's thyroiditis is more prevalent in areas with a high dietary iodinized salt intake, and smoking increases the risk (Huber *et al.*, 2008). Goiter can be seen on presentation, but thyroid atrophy is more common. Hashimoto's thyroiditis is associated with other endocrine diseases in polyglandular autoimmune failure syndrome (Addison's disease, type 1 *diabetes mellitus*, and

hypogonadism) (Huber *et al.*, 2008). The diagnosis is made by clinical features, elevated TSH, low thyroid hormone, and the presence of anti–thyroid peroxidase antibodies (anti-TPO) (Marcocci and Chiovato, 2000).

Graves' disease on the other hand, involves the binding of autoantibodies to TSH receptor which lead to stimulation. It is the most common cause of thyrotoxicosis (Brix *et al.,* 2001). Receptor activation stimulates thyrocyte growth and function (Ban and Tomer, 2003). The disease is more common in whites and Asians, and the incidence is lower in African Americans, and female-to-male ratio is 3.5:1 (Cooper, 2005). It is more common in patients with a family history of thyroid disease, especially Graves' disease. Graves' disease features include, swelling over the anterior shin (pretibial myxedema), thyroid eye disease (prominence of eyes, lid lag, globe lag, exophthalmos, lid edema, chemosis, and extraocular muscle weakness); and increased pigmentation and vitiligo. Thyroid ophthalmopathy is present in about 50% of Graves' patients. Smoking is a risk factor and therapeutic options include local measures to combat inflammation—glucocorticoids, plasmapheresis, and immune suppressants as well as orbital radiation, decompressive surgery, and thyroid ablation (Dorairajan *et al.,* 2002).

In Kenya, the available reports show that the prevalence of autoimmune thyroid disease is 2%, which ideally is for studies that were carried out between 1974 and 1978 (Kungu, 1974; Gitau, 1975). This indicates that there is paucity of data on the prevalence and etiology of the human autoimmune thyroid diseases. Thyroid dysfunction causes remarkable changes in glomerular and tubular functions and electrolyte and water homeostasis (Birewar *et. al.,* 2004). The most common thyroid conditions, and also the most common causes of thyroid hormone imbalances are autoimmune thyroid disorders, where auto antibodies attack thyroid gland (Nakazawa, 2008). In one form of autoimmune thyroid disorders called Hashimoto's

thyroiditis, the thyroid no longer produces hormones normally leading to hypothyroidism (Staecker *et. al.,* 2006). Hashimoto's disease is characterized by an underactive thyroid gland. Damage to the thyroid due to autoimmunity leads to decrease of thyroid hormone level in the blood, hormonal imbalance and inflammation of the gland (Ansen *et. al.,* 2005). Hypothyroidism can lead to other health complications such as goiter, poor memory, depression and others. In the opposite form, Graves' disease, the antibodies stimulate the gland and make the thyroid to produce too much T3and T4 hormones (Staecker *et. al.,* 2006). Over production of thyroid hormones, a condition known as hyperthyroidism can further aggravate a sufferer's health (Ansen *et. al.,* 2005). Often a genetic predisposition causes autoimmune disorders of the thyroid but many other factors like radiation or vitamin deficiencies and stress can trigger an autoimmune attack. Response of an individual's immune system to different triggers also plays a role in developing an autoimmune disease. The immune system is additionally affected by aging, chronic stress, hormonal imbalance and pregnancy and these conditions too can contribute to autoimmune diseases (Kumar, 2010).

What exactly causes the immune system to create these abnormal antibodies is still unexplained. Scientists have not yet identified the responsible gene for autoimmune thyroid disease but several genetic as well as environmental factors are recognized as triggers for the disease (Massoudi *et. al.,* 1995; Weetman, 2000). These include: stress, viral infections, exposure to toxins, use of steroids, iodine intake and female sex hormones.

The study was therefore designed to determine the histological types of thyroid diseases, thyroid hormones and patterns of presentation of thyroid disease, the association between pituitary and thyroid gland hormones, electrolytes, proteins and hematological indices in immunological thyroid diseases patients seeking treatment at the Moi Teaching and Referral Hospital (MTRH) which is located in the western part of Kenya.

1.2 Problem Statement

There are many thyroid pathologies in western Kenya but their current prevalence is not known. Thyroid antibody testing is not routinely available in developing countries and few studies have measured antibody levels in Africans. These included a retrospective review of records of 21,696 surgical operations examined at the University of Addis Ababa, which showed histopathological pattern of thyroid disease and their relationship with age and sex but test on antibodies were not done (Tsegaye, 2003). A retrospective analysis of 1494 thyroid cases seen at thyroid clinic of Kenyatta national hospital in Nairobi, Kenya, also showed histopathological pattern of thyroid disease and their relationship with age and sex but test on antibodies were not done (Kungu, 1974; Gitau, 1975). Another study in Nigeria showed the prevalence of thyroid antibodies (thyroperoxidase and thyroglobulin) (Okosieme *et al.,* 2006). Therefore in Africa only one study had done thyroid antibody testing. The significance of thyroid autoimmunity in African setting is still unclear. Thyroid antibodies are usually indicated when a patient has enlarged thyroid or symptoms suggesting thyroid dysfunction. Routine screening of thyroid is usually accomplished using thyroid tests such as T3, T4 and TSH. The specificity and sensitivity of the thyroid antibody testing is improving although there are many distinct methodologies and each has different reference ranges. In Kenya, where the mode of treatment to thyroid disorders is mainly thyroidectomy, documentation of case patterns, prevalence and aetiology is still very poor (Kungu, 1974; Gitau, 1975). Thyroid dysfunction causes remarkable changes in glomerular and tubular functions and electrolyte and water homeostasis (Birewar *et al.,* 2004). Therefore, there is need to correlate between blood electrolytes, serum proteins, hematological profiles and thyroid function in immunological thyroid diseases patients. If indeed the profiles are altered, this would be a useful pointer to immunological thyroid diseases patients since laboratory diagnosis of these conditions are made based on the thyroid hormone levels only.

Therefore, this study was carried to determine the prevalence, histological patterns, thyroid hormones and autoantibody levels in immunological thyroid disease patients at Moi Teaching and Referral Hospital (MTRH) in western Kenya and show whether autoimmune thyroid diseases are rare in MTRH or cases are under reported due to the absence of appropriate diagnostic techniques.

1.3 Study Objectives

1.3.1 Broad objective

To determine the prevalence and factors associated with immunological thyroid disease among thyroid disorder patients at Moi Teaching and Referral Hospital (MTRH), Western Kenya.

1.3.2 Specific objectives

1. To determine the prevalence and histological patterns of immunological thyroid diseases in patients undergoing thyroidectomy at the Moi Teaching and Referral Hospital (MTRH) in western Kenya.
2. To compare between pituitary and thyroid gland hormones (TSH, T3, T4) and thyroid autoantibody levels in immunological thyroid diseases patients undergoing thyroidectomy at the Moi Teaching and Referral Hospital (MTRH), western Kenya.
3. To determine the levels of blood electrolytes, proteins and hematological indices in immunological thyroid diseases patients undergoing thyroidectomy at the Moi Teaching and Referral Hospital (MTRH), western Kenya.

1.4 Research Questions

1. What is the prevalence and histological patterns of immunological thyroid diseases among patients undergoing thyroidectomy at the Moi Teaching and Referral Hospital (MTRH) in western Kenya?
2. How do pituitary and thyroid gland hormones (TSH, T3 and T4) levels compare with the thyroid autoantibody levels in immunological thyroid diseases among patients undergoing thyroidectomy at the Moi Teaching and Referral Hospital (MTRH) in western Kenya?
3. What are the levels of blood electrolytes, proteins and hematological indices in immunological thyroid diseases patients undergoing thyroidectomy at the Moi Teaching and Referral Hospital (MTRH), western Kenya?

1.5 Justification of the Study

Studies of thyroid antibodies in African patients have been few to date and most instances have been performed with agglutination or immune fluorescence methods (Okosieme *et al.*, 2006), which suffer from poor sensitivity and specificity. These methods have since been rendered obsolete by the advent of more sensitive and specific enzyme-linked immunosorbent assay (ELISA) techniques. Therefore, it remains unclear whether autoimmune thyroid disorders are indeed rare in Africans or if cases are overlooked due to the absence of appropriate diagnostic facilities. The latter possibility has implications for patient well-being, since undetected autoimmune thyroid disease carries considerable morbidity. There is also need to correlate between the thyroid function tests and thyroid immunological diseases. If indeed there exist a correlation between them, this would be a useful pointer to immunological thyroid diseases since laboratory diagnosis of immunological thyroid disease is expensive. Only the suspected cases were subjected to immunological diagnosis.

1.6 Significance of the Study

This study helped to diagnose an autoimmune thyroid disease and to separate it from other forms of thyroiditis. Briefly, it investigated the cause of goiter and thyroid dysfunction in western part of Kenya. The research findings provided knowledge of autoimmune thyroid disease to the community at large and establish the prevalence of thyroiditis at MTRH, therefore suggest preventive measures to the affected. This research formed the basis for the prevalence studies in other parts of Kenya.

CHAPTER TWO

2.0 LITRATURE REVIEW

This section gives an analysis of existing information which is relevant to the topic under study and also seeks to identify gaps in knowledge that need attention or further research.

2.1. Prevalence and Histological Patterns of Immunological Thyroid Disease Patients

2.1.1 Autoimmunity

Autoimmunity is defined as an immune response directed against self antigens (Furugaki *et al.*, 2004). It does not distinguish whether the response is innate or acquired and, if acquired, whether it is induced by a foreign or autologous antigen; it is also not restricted to a T-cell or B-cell response, and only requires that the immune response be directed to a self-antigen (Rose, 1997; Ueda *et al.*, 2003). Autoimmune disease is a pathologic condition caused by an autoimmune response. However, these definitions can be unclear since it is frequently difficult to assign causality when dealing with a human disease. It is useful, therefore, to consider the evidence of an autoimmune aetiology of a human disease with three degrees of stringency (Davidson and Diamond, 2001).

To determine whether autoimmunity is the cause of the disease rather than an accompanying feature or a consequence, the demonstration of autoantibodies is the first step in the diagnosis of these diseases, although the antibodies may not be the actual pathogens of the disorder (Arbuckle *et al*, 2003). Naturally occurring autoantibodies are common in all immunologically competent people and may even rise nonspecifically during the course of disease or injury (Marcocci and Chiovato, 2000). Thus, the mere presence of autoantibodies does not necessarily establish a cause-and-effect relationship, since the autoantibodies may be the result, not the cause, of the disease process. The presence of autoantibody responses has

great value in diagnosis and prognosis of thyroid diseases (Terry *et al.*, 2009). Autoantibodies may be present many years before the diagnosis of diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, antiphospholipid syndrome, and type *1 diabetes mellitus*. Combined with genetic information or family history, the presence of autoantibodies may be highly predictive of the later onset of an autoimmune disorder (Arbuckle *et al.*, 2003; Rose, 2008).

2.1.2 Epidemiology of autoimmune thyroid diseases

Autoimmune thyroid disease is a common organ specific autoimmune disorder affecting mostly the middle aged women. About 2 to 4 percent of women and up to 1% of men are affected worldwide and the prevalence rate increases with advancing age (Canaris *et al.*, 2000; Deshun *et al.*, 2009). Autoimmune thyroid disease is the major cause of excess new hormone synthesis by the thyroid gland. Graves' disease is the most common cause of hyperthyroidism (Brent, 2008). Hyperthyroidism is more common in women than men (5:1 ratio) (Brent, 2008). The overall prevalence of hyperthyroidism, which is approximately 1.3 percent, increases to 4 to 5 percent in older women (Hollowell *et al.*, 1994). Hyperthyroidism is also more common in smokers (Asvold *et al.*, 2007). Graves' disease is seen most often in younger women, while toxic nodular goiter is more common in older women (Holm, 2005; Nuria *et al.*, 2009). In one prospective cohort study of adult women, the overall incidence of Graves' disease was 4.6 per 1000 during 10 years of observation (Holm, 2005).

Cancer of the thyroid (thyroid follicular carcinoma) is the most common endocrine malignancy. Some 5-10% of patients with thyroid cancer will die of their disease. Thyroid neoplasms arising from follicular cells (adenoma, carcinoma, and follicular/papillary carcinoma) show a broad range of overlapping clinical and cytologic features. A clear distinction between benign and malignant disease based solely on cytological examination of a needle biopsy specimen may be difficult. For this reason, a surgical procedure to remove all

or a large portion of the thyroid gland may be necessary to obtain sufficient tissue for a definitive diagnosis of follicular thyroid cancer. Pathological examination showing capsular or vascular invasion may be required for this determination (Canaris *et al.*, 2000). The major risk factors are exposure to ionizing radiation. Diseases such as Hashimoto's thyroiditis and nodular colloid goitre have been implicated as some of the predisposing factors (Arata *et al.*, 2006).

"Hashitoxicosis" (a neologism that combines Hashimoto and thyrotoxicosis) is a term used to describe rare patients with autoimmune thyroid disease who initially present with hyperthyroidism and a high radioiodine uptake caused by TSH-receptor antibodies similar to Graves' disease (Fatourechi *et al.*, 1971). This is followed by the development of hypothyroidism due to infiltration of the gland with lymphocytes and resultant autoimmune-mediated destruction of thyroid tissue similar to chronic lymphocytic thyroiditis (Hashimoto's thyroiditis) (Arata *et al.*, 2006).

Toxic adenoma and toxic multinodular goiter are the result of focal and/or diffuse hyperplasia of thyroid follicular cells whose functional capacity is independent of regulation by TSH. Activating somatic mutations of the genes for the TSH receptor have been identified in both toxic adenomas and nodules of toxic multinodular goiters (Duprez *et al.*, 1997; Parma *et al.*, 1997; Holzapfel *et al.*, 1997). The mutations are usually in the transmembrane domain of the receptor, but can be in the extracellular domain (Führer *et al.*, 1997; Kopp *et al.*, 1997). The mutant receptors activate adenylyl cyclase in the absence of TSH (Prabhakar *et al.*, 1997). Toxic multinodular goiter tends to be more common in areas where iodine intake is relatively low (Wolde-gabriel *et al.*, 1992). In comparison, the frequency of thyroid adenomas is not related to iodine intake (Wolde-gabriel *et al.*, 1992). Adenomas are second most common after colloid goitre (Namba *et. al.*, 1990). An adenoma is a benign tumor formed by the autonomous proliferation of follicular cells within the thyroid lobe. The adjacent thyroid cells

are compressed in varying degrees, depending on the size of the adenoma. Adenoma usually do not have a complete fibrous capsule but large adenomas may be partially encapsulated by a thin layer of fibrous tissue (Vaidya, 2002). The neoplastic cells are often more hyperchromatic than the surrounding normal follicular cells and form variable size colloid-containing follicles, larger cystic spaces or solid sheets. Neoplastic cells may form papillary structures with cells varying from cuboidal to low columnar. The nucleus to cytoplasmic ratio is often high. There is no evidence of vascular invasion. Endocrinologically-active thyroid, adenomas result in the colloid involution of follicles in the rim of the surrounding thyroid due to the inhibition of TSH secretion by elevated blood T4 and T3 levels. Follicular adenomas are clonal (Namba *et. al.,* 1990). Point mutations in the H-ras, K-ras, and N-ras proto-oncogenes have been identified in follicular adenomas, because defects in both alleles of a tumor suppressor gene are needed for tumor formation (Namba *et. al.,* 1990).

The term thyroiditis has been applied to a group of heterogeneous disorders that result in inflammation of thyroid tissue with transient hyperthyroidism due to release of preformed hormone from the colloid space (Volpé *et al.,* 1979). This initial presentation is followed by a hypothyroid phase and then recovery of thyroid function. When the term subacute thyroiditis is used without modification, it usually refers to subacute granulomatous thyroiditis which is a viral or post-viral syndrome characterized by fever, malaise, and an exquisitely painful and tender goiter (Volpé *et al.,* 1979). In comparison, painless thyroiditis (silent thyroiditis or subacute lymphocytic thyroiditis) is part of the spectrum of autoimmune thyroid diseases (Nikolai *et al.,* 1982) and has a particular proclivity to occur in the postpartum period (postpartum thyroiditis) (Roti *et al.,* 1992). Patients with thyroiditis usually have a radioiodine uptake of less than one percent, making radioiodine therapy impossible as well as inappropriate. Acute suppurative thyroditis (AST) leading to thyroid abscess is a rare clinical entity. Acute suppurative thyroditis affects especially patients with pre-existing thyroid gland

pathology and in childhood it is associated with local anatomic defects. Because of its rarity and unusual clinical features, the diagnosis of thyroid abscess is often delayed. Thyroid abscess is infrequently encountered condition with a rarity that is attributed to anatomic and physiologic characteristics of the gland that imparts a unique quality of infection of resistance (Lester, 1976). When discovered, a thyroid abscess presents as acutely painful swelling. The differential diagnosis for a painful thyroid is limited, with sub-acute and chronic thyroiditis being the most often encountered process (Mengistu, 1992).

In one study, the prevalence of thyroglobulin antibody (TgAb) and thyroperoxidase antibody (TPOAb) in various categories of patients and in healthy adults were found in 4% and 7%, respectively, of healthy adult controls, 11.6% and 76.8% of patients with Graves' disease (GD), 25% and 12.5% of patients with toxic nodular goiter (TNG) and 9.52% and 14.29% of patients with simple non-toxic goiter (SNTG). In these, TPOAb testing confirmed Hashimoto's thyroiditis (HT) in 6 patients, and identified 2 further cases that would have been misdiagnosed without antibody testing (Okosieme *et al.*, 2006).

Retrospective review of records examined at the University of Addis Ababa, showed histopathological pattern of thyroid disease and their relationship with age and sex. Females were most commonly affected by thyroid diseases (80.5%) than males (19.5%) (Tsegaye, 2003). A total of 21,696 surgical operations were submitted for histological examination during the study period. Out of these, 792 (3.6%) were thyroid specimens. Nodular colloid goiter accounted for 600 (76.9%) cases. Thyroid neoplasms were encountered in 164 (21%) cases only. The benign tumor (adenoma) was seen in 100 (12.8%) cases and thyroid carcinoma in 64 (8.2%) cases. Thyroiditis constituted 16 (2.1%) of the cases (Tsegaye, 2003).

Retrospective analysis of 1494 thyroid cases seen at the thyroid clinic of Kenyatta National Hospital in Kenya, reported that females (80.5%) with thyroid diseases were more than males

(19.5%) (Gitau, 1975). This was in agreement with other studies done in Ethiopia (Mekones, 1996; Wolde-gabriel *et al.,* 1992).

2.1.3 Etiology

The etiology of AITD is multifactorial. Susceptibility to the disease is determined by a combination of immune mechanism, genetics, environmental (iodine, infection and stress) and constitutional factors.

2.1.3.1 Immune mechanisms

A variety of immune mechanisms may be involved in the pathogenesis of Graves' hyperthyroidism. The major mechanisms for which there is some evidence are molecular mimicry (specificity crossover), thyroid-cell expression of human leukocyte-associated (HLA) molecules (antigens), and bystander activation (Terry *et al.,* 2009; Hanafusa *et al.,* 1983; Tomer and Huber, 2009).

2.1.3.1. a Molecular mimicry

Molecular mimicry implies structural similarity between some infectious or other exogenous agent and human proteins, such that antibodies and T cells activated in response to the exogenous agent react with the human protein, in this instance one or more thyroid proteins. As an example, in an analysis of 600 monoclonal antibodies raised against a large variety of viruses, 4 percent of the monoclonal antibodies cross-reacted with uninfected tissues (Srinivasappa *et al.,* 1986; Bayliss, 1982; Cotran *et al.,* 1994; Kabel *et al.,* 1988).

2.1.3.1. b Thyroid cell abnormal expression of HLA II molecules

Thyroid epithelial cells from patients with autoimmune thyroid disease (including Graves' disease) but not normal subjects express MHC class II molecules, notably HLA-DR molecules (Bottazzo *et al.,* 1983). This expression could be the direct result of viral or other

infections of thyroid epithelial cells, or it may be induced by cytokines such as interferon gamma produced by T cells that have been attracted to the gland either by an infection or directly because of the presence of thyroid antigens (Bottazzo *et al.,* 1983; Pei-Win *et al.,* 2004).

Class II molecule expression provides a mechanism for presentation of thyroid antigens to and activation of autoreactive T cells, with the potential for persistence of thyroid disease (Furugaki *et al.,* 2004). Several experimental observations provide support for this hypothesis: induction of class II molecules on thyroid epithelial cells by interferon gamma can induce autoimmune thyroiditis in susceptible mice (Neufeld *et al.,* 1988); viruses can directly induce class II molecule expression on thyroid cells, independent of cytokine secretion (Neufeld *et al,* 1988; Khoury *et al.,* 1991; Pei-Win *et al.,* 2004); thyroid epithelial cells expressing class II molecules can present viral peptide antigens to cloned T cells (Kimura, 1991); thyroid antigen-specific T cell clones in normal rats react specifically with cloned autologous thyroid cells in the absence of more conventional antigen-presenting cells (Londei, 1984); and an animal model of Graves' disease induced by cells expressing the TSHR is only effective when the cells also express MHC class II antigens (Shimojo *et al.,* 1996; Kita, 1999). These findings strongly support the view that an insult, such as infection, may induce class II molecule expression on human thyroid cells and that these cells then may act as antigen-presenting cells to initiate an autoimmune response (Pei-Win *et al.,* 2004).

The expression of a T cell co-stimulator molecule, CD40, on thyroid epithelial cells indicates that co-stimulatory molecules are available for this action (Takashi *et al.,* 2009). In addition, intrathyroidal dendritic cells and B cells may also serve as potent antigen-presenting cells (Abbas *et al.,* 1996; Takashi *et al.,* 2009). The description of hyperthyroidism in mice immunized with fibroblasts co-expressing class II molecules and human TSH receptors

provides further evidence that cells need not be "professional" antigen-presenting cells to present antigen so long as they can acquire the ability to express class II molecules (Martin *et al.*, 1999).

2.1.3.4 Bystander activation

In order for HLA class II antigen expression and presentation of antigens to be realized, there must be a local insult to initiate the responses. As mentioned above, this may take the form of a direct insult to the thyroid by a viral infection of the thyroid cells or of immune cells. Even the arrival of activated T cells within the thyroid gland may perhaps initiate such a series of events in a susceptible subject with the appropriate immune repertoire (Horwitz *et al.*, 1998). Evidence show that such bystander activation of local T cells, which may not be thyroid specific, may exert via cytokines a marked activation effect on resident thyroid-specific T cells. Evidence for such bystander effects has been obtained in an animal model of viral induced autoimmune insulitis and in experimental autoimmune thyroiditis (Arata *et al.*, 2006).

2.1.4 Precipitating and predisposing factors for Graves' disease

Several factors that predispose to or initiate Graves' hyperthyroidism have been proposed and include genetic susceptibility, infection, stress, sex steroids, smoking, pregnancy and drugs as reviewed in the sections that follows (Yuji, 2007).

2.1.4.1 Genetic susceptibility

There is abundant epidemiologic evidence for genetic susceptibility to Graves' hyperthyroidism and chronic autoimmune thyroiditis (Brix *et al.*, 2001; Stelios *et al.*, 2008). The diseases cluster in families and are more common in women. The concordance rate in monozygotic twins is 20 to 40 percent (Stenszky *et al.*, 1985; Tomer *et al.*, 2003; Tomer and Davies, 2003). The sibling recurrence rate for Graves' disease exceeds 10.0 (Villanueva *et al.*,

2003). There is an association between autoimmune thyroid disease and certain alleles of cytotoxic T lymphocyte-antigen/associated protein 4 (CTLA-4). As an example, in one study of 379 patients with Graves' hyperthyroidism in the United Kingdom, 42 percent had a particular allele (G allele) of the CTLA-4 gene, as compared with 32 percent of 363 normal subjects (Tomer and Davies, 2003). There is an association with certain alleles of HLA on chromosome 6. As an example, a study of Caucasian patients in North America found that HLA-DRB1*08 and DRB3*0202 were associated with the disease and that DRB1*07 was protective (Chen *et al.*, 1999; Vaidya *et al.*, 2002; Hadj-Kacem *et al.*, 2009).

2.1.4.2 Infection

If infection were the cause of Graves' hyperthyroidism, an identifiable agent should be present in the majority of patients and it should be possible to induce the disease by transferring the agent. Possible infections of the thyroid gland itself (subacute thyroiditis and congenital rubella) have been associated with thyroid autoimmune disease and could initiate class II molecule expression (Humphrey *et al,* 1991). Hepatitis C infection is a well recognized precipitator of autoimmune thyroid disease when treated with interferon therapy. There is, however, no evidence that these or any other infections or exposures lead directly to autoimmune thyroid disease (Humphrey *et al,* 1991; Neumann-Haefelin, 1993; Tomer and Davies, 1993).

2.1.4.3 Stress

As compared with normal subjects or patients with toxic nodular goiter, patients with Graves' hyperthyroidism more often give a history of some type of psychologic stress in particular negative life events such as loss of a spouse before the onset of their hyperthyroidism (Sonino *et al.*, 1993; Kung *et al.*, 1995; Matos-Santos, 2001). In general, stress appears to induce a state of immune suppression, possibly mediated by the actions of cortisol on immune cells.

Suppression of stress may be followed by rebound immunologic hyperactivity. Such a response could precipitate autoimmune thyroid disease in genetically susceptible subjects (Sonino *et al.*, 1993; Kung *et al.*, 1995; Matos-Santos, 2001; Karlsson, 1991).

2.1.4.4 Sex steroids

More women develop Graves' hyperthyroidism than men, with a ratio of approximately 7:1, an effect that is often said to be mediated in some way by more estrogen or less testosterone (Yin *et al.*, 2007). There is a large body of evidence that moderate amounts of estrogen enhance immunologic reactivity to self-antigens (Kincade *et al.*, 1994; Da Silva, 1995). However, it is just as likely that the X chromosome is the source of the enhanced susceptibility rather than sex steroids since the susceptibility continues after the menopause. For example, X-chromosome inactivation has been associated with autoimmune thyroid disease (Yin *et al.*, 2007).

2.1.4.5 Smoking

Smoking is a risk factor for Graves' hyperthyroidism (relative risk approximately 2.0) and an even stronger risk factor for Graves' ophthalmopathy (Bartalena *et al.*, 1989; Prummel *et al.*, 1993; Holm *et al.*, 2005).

2.1.4.6 Pregnancy

Graves' disease is uncommon during pregnancy because hyperthyroidism is associated with reduced fertility and increased pregnancy loss. In addition, pregnancy is a time of immune suppression so that the disease tends to improve as pregnancy progresses. During pregnancy, both T-cell and B-cell functions are diminished, and the rebound from this immunosuppression may contribute to the development of postpartum thyroid disease (Glinoer, 2004).

It has also been suggested that fetal microchimerism (the presence of fetal cells in maternal tissue) might play a role in the development of postpartum autoimmune thyroid disease (Ando *et al.*, 2003). Up to 30 % of young women give a history of pregnancy in the 12 months before the onset of Graves' disease, indicating that postpartum Graves' disease is a surprisingly common presentation and that pregnancy is a major risk factor in susceptible women (Jansson *et al.*, 1987).

2.1.4.7 Drugs

Iodine and iodine-containing drugs such as amiodarone may precipitate Graves' disease, or a recurrence of Graves' disease, in a susceptible individual (Noel *et al.*, 2002; Bartalena *et al.*, 2002). Iodine is most likely to precipitate thyrotoxicosis in an iodine deficient population simply by allowing the TSHR-Ab to be effective in stimulating production of thyroid hormone. Whether there is any other precipitating event is unclear. Iodine and amiodarone may also damage thyroid cells directly and release thyroid antigens to the immune system (Benbassat *et al.*, 2000).

2.2. Predisposing and Precipitating Factors for Hashimoto's Thyroiditis

Infection, stress, sex steroids, pregnancy, iodine intake, and radiation exposure are the known possible precipitating factors for Hashimoto's thyroiditis (Walsh *et al.*, 2006). Fetal microchimerism within the maternal thyroid is also a possibility (Srinivasappa *et al.*, 1988; Bendtzen *et al.*, 1989; Walsh *et al.*, 2006; Ando *et al.*, 2003; Strieder *et al.*, 2003).

2.2.1 Genetic susceptibility

There is genetic susceptibility to Hashimoto's thyroiditis and much has been learned in recent years concerning the susceptibility genes for this disorder in particular and for autoimmune thyroid disease in general (Tomer *et al.*, 1999). Several studies have shown evidence for

genetic susceptibility to Hashimoto's thyroiditis. The disease clusters in families, sometimes alone and sometimes in combination with Graves' disease (Tamai *et al.*, 1980). The sibling recurrence risk is >20 (Villanueva *et al.*, 2003). The concordance rate in monozygotic twins is 30 to 60% despite random combinations of T cell receptor and antibody V genes at the time of recombination (Brix *et al.*, 2000). There is an association, albeit relatively weak, with certain HLA alleles such as DR3. There is linkage to certain alleles of the gene for CTLA-4. The thyroglobulin gene has been linked to autoimmune thyroid disease and has been suggested to code for Tg forms with different immune reactivity (Ban *et al.*, 2003).

2.2.2 Infection

No infection is known to cause or even be closely associated with Hashimoto's thyroiditis in humans (Tomer *et al.*, 1993), although thyroiditis can be induced in experimental animals by certain viral infections (Srinivasappa *et al.*, 1988). Patients with subacute granulomatous thyroiditis (presumed to be a viral infection) and congenital rubella may have thyroid antibodies for a few months after their illnesses, and the infections could initiate expression of MHC class II molecules in the thyroid gland. However, neither disorder is known to be commonly followed by chronic thyroiditis although evidence of thyroid autoimmunity may persist (Weetman *et al.*, 1987).

2.2.3. Stress

Stress of various types has been linked to Hashimoto's thyroiditis. The proposed mechanisms include induction of immune suppression by non-antigen-specific mechanisms, perhaps due to the effects of cortisol or corticotropin-releasing hormone on immune cells, followed by immune hyperactivity leading to autoimmune thyroid disease (Bendtzen *et al.*, 1989).

2.2.4 Sex steroids and pregnancy

More women than men have Hashimoto's thyroiditis, suggesting a role for sex steroids. However, older women may be more likely to have Hashimoto's thyroiditis than younger women, suggesting that the presence or absence of estrogen may not be the important factor (Gause *et al.*, 1986).

Another possible explanation for female predominance is skewed X-chromosome inactivation, which was found in 34 percent of female twins with autoimmune thyroid disease and only 11 percent of controls (Brix *et al.*, 2005; Yin *et al.*, 2007). It is possible that the self-antigens on the inactivated X-chromosome might not be expressed sufficiently to allow tolerance. During pregnancy, there is a marked increase in CD4+CD25+ regulatory T cells which lead to diminished function of both T-cells and B-cells and the rebound from this immunosuppression is thought to contribute to the development of postpartum thyroiditis (Raghupathy, 1997). Pregnancy-associated immune suppression is associated with a shift to Th_2 T cells and a shift in cytokine profiles (Raghupathy, 1997).

A variety of local factors at the immune cell-trophoblast interface are also known to be important modulators of immune function in pregnancy. The trophoblast cells located in the placenta, and subject to maternal immune surveillance, serve as physical barriers between mother and fetus, and have been shown to express several immune modulating molecules, such as HLA-G, FasL, and indoleamine 2, 3-dioxygenase as well as secreting a variety of cytokines (Davies, 1999). HLA-G is one of the members of the MHC class I family and is known to inhibit natural killer cell function and dendritic cell maturation. Fas ligand interacts with Fas antigen and induces apoptotic cell death of fetal antigen-reactive maternal lymphocytes. Indoleamine 2, 3-dioxygenase, which catalyzes tryptophane in lymphocytes, has proven to be critical in the maintenance of allogeneic pregnancy in mouse (Weetman, 1999).

Other than these local modulators, progesterone produced by the placenta affects cytokine profiles across the whole maternal immune system. Approximately 20 percent of patients with postpartum thyroiditis go on to develop classical Hashimoto's disease in later years (Othman *et al.*, 1990).

2.2.5 Iodine intake

Mild iodine deficiency is associated with a lower prevalence of Hashimoto's disease and hypothyroidism, while excessive intake is associated with a higher prevalence (Gbadebo *et al.*, 2005). As an example, in China, autoimmune thyroiditis was found in 0.3 percent of those with mildly deficient iodine intake and 1.3 percent of those with excessive iodine intake (Walsh *et al.*, 2006).

2.2.6 Radiation exposure

Following the tragic Chernobyl nuclear accident, the exposed children developed a high frequency of thyroid autoantibodies (Pacini *et al.*, 1998). All the evidence suggests that the presence of thyroid antibodies increases the risk of developing thyroid dysfunction (Vanderpump *et al.*, 1995; Huber *et al.*, 2002). Whether background radiation to which we are all exposed has any role in susceptibility to autoimmune thyroid disease is unknown. In a population based study of 4299 subjects, 160 had an occupational exposure to ionizing radiation, nearly 60 percent of the subjects worked in a nuclear power plant, while the rest were either medical or laboratory workers. Ten percent of the female subjects with radiation exposure met criteria for autoimmune thyroid disease (anti-TPO antibodies greater than 200 IU/ml and hypoechogenicity on ultrasound) compared to 3.4 percent of those without an exposure. Subjects with greater than five years of exposure to ionizing radiation were particularly at high risk (Volzke *et al.*, 2005).

2.2.7 Fetal microchimerism

Fetal cells have been identified within maternal thyroid glands in patients with autoimmune thyroid disease. Such cells may initiate graft versus host reactions with the thyroid gland and play a significant role in the development of Hashimoto's thyroiditis. To date, however, this remains hypothetical (Ando *et al.,* 2003; Klintschar, 2001; Srivatsa *et al.,* 2001; Imaizumi *et al.,* 2002).

2.3. Association between Pituitary, Thyroid Gland Hormones and Thyroid Autoantibodies

2.3.1 Thyroid gland diseases pathology and pathogenesis

Autoimmune thyroid diseases are conditions where the immune system attacks the thyroid gland. The thyroid gland is a butterfly-shaped organ that is found in the anterior of the neck. Thyroid hormone helps control the body physiological process such as; heart rate, growth and normal body temperature (Tsegaye *et.al.,* 2003; Giordano *et al.*, 1997). Thyroid hormones also control how the body uses energy and affects weight gain and loss. Autoimmune thyroid disorders often affect people between 20 and 50 years of age especially women. Having a family member with autoimmune disease may also increase the risk. Having too much or little dietary iodine, stress, infections and heavy smoking may cause autoimmune diseases (Tomer and Huber, 2009).

2.3.2 Autoimmune thyroiditis

Autoimmune thyroiditis is associated with two diseases that affect the thyroid. These diseases include Hashimoto thyroiditis and Graves' disease (Tomer and Huber, 2009). Hashimoto's thyroiditis is a chronic inflammatory autoimmune disease of the thyroid gland. The thyroid gland produces two hormones, T3 and T4, which control metabolism of almost all cells in the body. The pituitary gland secrets a hormone called thyroid stimulating hormone (TSH), which

increases thyroid gland hormone production. Hashimoto's thyroiditis occurs when inflammation caused by an autoimmune process destroys the thyroid gland, leading to an insufficient production of thyroid hormones. Symptoms are usually painless, diffuse and gradual enlargement of the thyroid gland, which can be noticed as enlargement of neck (Daniela, 2001). Rarely, it can be accompanied with shortness of breath (dyspnea) or difficulty swallowing (dysphagia) due to the pressure of the growing goiter. The thyroid hormone deficiency may have no symptoms. However, the common symptoms are: fatigue, depression, sensitivity to cold, weight gain, muscle weakness, coarsening of the skin, dry or brittle hair, constipation, muscle cramps, increased menstrual flow, and increased risk of miscarriage (Daniela, 2001).

The incidence rate of autoimmune thyroiditis are 0.3–1.5 cases per 1,000 per year. The female-to-male ratio is 20:1 children (Tomer and Huber, 2009). The disease is most common in middle aged women, but it can affect all age groups, including children (Tomer and Huber, 2009). Currently, there is no treatment capable of stopping the autoimmune process leading to Hashimoto's thyroiditis. Hypothyroidism, which is a result of the thyroid gland destruction, can be treated by a lifelong thyroid hormone replacement. Under the hormone replacement therapy, the size of the goiter usually decreases, if not, surgery may be required (Daniela, 2001; Tomer and Huber, 2009).

2.3.3 Graves' disease pathology and pathogenesis

Graves' disease is part of a syndrome that consists of hyperthyroidism, goiter, ophthalmopathy (orbitopathy) and occasionally a dermopathy referred to as pretibial or localized myxedema. The terms Graves' disease and hyperthyroidism are not synonymous, because some patients have ophthalmopathy but no hyperthyroidism, and there are many other causes of hyperthyroidism in addition to Graves' disease (Wartofsky *et al.*, 1998; Sonino *et al.*, 1993).

Hyperthyroidism is the most common feature of Graves' disease and is caused by autoantibodies to the thyrotropin receptor (TSH-R) that activate the receptor, thereby stimulating thyroid hormone synthesis and secretion as well as thyroid growth (causing a diffuse goiter) (Mekones, 1996). The presence of TSHR-Ab in serum and ophthalmopathy on clinical examination distinguishes the disorder from other causes of hyperthyroidism and diffuse goiter (Mekones, 1996; Wolde-gabriel *et al.*, 1992).

The histology of the thyroid gland in patients with Graves' hyperthyroidism is characterized by follicular hyperplasia, a patchy (multifocal) lymphocytic infiltration and rare lymphoid germinal centers (Paschke *et al.*, 1991). The majority of intrathyroidal lymphocytes are T cells. This means that in Graves'disease, T lymphocytes are the main cells involved in thyroid-cell stimulation and B cells are much less common than in chronic autoimmune thyroiditis (Hashimoto's disease). Thyroid epithelial cell size correlates with the intensity of the lymphocytic infiltrate, suggesting thyroid-cell stimulation by local B cells secreting TSHR-Ab (Terry *et al.*, 2009; McIver and Morris, 1998; Paschke *et al.*, 1991; Wu *et al.*, 1994).

2.3.4 Hashimoto's disease pathology and pathogenesis

Hashimoto's thyroiditis (chronic autoimmune thyroiditis) is the most common cause of hypothyroidism in iodine-sufficient areas of the world (Terry *et al.*, 2009). Thyroid failure is seen in up to 10 percent of the population and its prevalence increases with age (Giordano *et al.*, 1997). It is characterized clinically by gradual thyroid failure, goiter formation, or both, due to autoimmune-mediated destruction of the thyroid gland involving apoptosis of thyroid epithelial cells (Wartofsky *et al.*, 1998). Nearly all patients have high serum concentrations of antibodies against one or more thyroid antigens, diffuse lymphocytic infiltration of the thyroid, which includes predominantly thyroid-specific B and T cells, and follicular

destruction (Hollowell *et al.*, 1994; Wartofsky *et al.*, 1998; Aozasa *et al.*, 1989). The cause of Hashimoto's thyroiditis is thought to be a combination of genetic susceptibility and environmental factors. Hashimoto's thyroiditis is primarily a disease of women, with a sex ratio of approximately 7:1; it can also occur in children (Terry *et al.*, 2009). Variant mild forms of Hashimoto's thyroiditis include silent (or painless) thyroiditis and postpartum thyroiditis, both of which are transient but may be followed years later by thyroid failure (Giordano *et al.*, 1997).

Hypothyroidism is the characteristic functional abnormality; the inflammatory process early in the course may involve enough apoptosis to cause thyroid follicular disruption and thyroid hormone release, causing transient hyperthyroidism sometimes referred to as Hashitoxicosis (Fatourechi *et al.*, 1971; Fatourechi *et al.*, 1912). Some of these patients have a transiently increased radioiodine uptake, and rare patients may cycle between hypothyroidism and Graves' disease, perhaps secondary to alternating production of thyrotropin (TSH) receptor blocking and stimulating antibodies (Kraiem, 1992; Takasu *et al.*, 1990). The usual course of Hashimoto's thyroiditis is gradual loss of thyroid function. Among patients with this disorder who have mild (subclinical) hypothyroidism, exhibited as slight increases in TSH and the presence of thyroid antibodies, overt hypothyroidism occurs at a rate of about 5 percent per year (Vanderpump *et al.*, 1995; Huber *et al.*, 2002). Mechanisms of thyroid autoimmunity with an emphasis on Hashimoto's thyroiditis include; molecular mimicry, bystander activation and thyroid cell HLA antigen expression (Hollowell *et al.*, 1994).

2.3.5 Autoimmune features

All forms of thyroid autoimmunity are associated with a lymphocytic infiltrate in the thyroid. These lymphocytes are largely responsible for generating both T and B cell-mediated autoreactivity. Other sites such as thyroid draining lymph nodes and bone marrow may also contain thyroid autoreactive lymphocytes in AITD. The initial autoimmune response by CD4+ T cells appears to upregulate the

secretion of interferon-gamma resulting in enhanced expression of MHC II molecules on thyrocytes. This most likely triggers expansion of autoreactive T cells and gives rise to the characteristic inflammatory response and as the disease progress, thyrocytes are targeted for apoptosis resulting in hypothyroidism. Another contributing factor to the observed hypothyroidsm in Hashimoto's thyroiditis patients could be the circulating TSH inhibitory antibodies. Graves' disease on the other hand represents the other end of spectrum wherein the patients suffer from hyperthyroidism. The activation of thyroid specific CD4+ T cells leads to the recruitment of autoreactive B cells and the mounting of thyroid stimulatory immune response via anti-thyroid antibodies (Prasad *et al.,* 2003).

2.3.6 Autoantibodies

2.3.6.1 Thyroid peroxidase (TPO) antibodies

Thyroid peroxidase (TPO) antibodies is the key thyroid enzyme catalyzing both the iodination and coupling reaction for the synthesis of thyroid hormone. It is membrane bound and found in the cytoplasm and in high concentration on the apical microvillar surface of thyrocytes. It's molecular weight is between 100 to 105-kDa and previously was known as thyroid microsomal antigen (McLachian *et al.,* 1992). Multiple T and B cell epitopes exists within the molecule and the antibody response to TPO is restricted at the level of the germ line heavy and light chain variable (V) region (Mclntosh *et al.,* 1998).

Anti-TPO autoantibodies are found in over 90% of patients with autoimmune hypothyroidism and Graves' disease. Together with thyroglobulin (TG) antibodies, these are the predominant antibodies in autoimmune hypothyroidism (AH). Anti-TPO antibodies are mainly of the IgG class 1 and IgG4 subclasses in excess (Njemini *et al.,* 2002; Silva *et al.,* 2003; Hawa *et al.,* 2006).

2.3.6.2 Thyroglobulin (TG) antibodies

Thyroglobin (TG) is a 660-kDa glycoprotein composed of two identical subunits of 330 kDa each. It is secreted by the thyroid follicular cells into the follicular lumen and stored as a colloid substance within the thyroid follicles. Each TG molecule has around 100 tyrosine residues, a quarter of which are iodinated. These residues couple to triiodothyronine (T3) and thyroxine (T4). When TSH stimulates the thyroid cell, TG is endocytosed and hydrolyzed in lysosome releasing T3 and T4. The exact location of T and B cell epitopes within TG is uncertain (Canayanniotis *et al.*, 1997).

Thyroglobulin autoantibodies are found in less than 60% of patients with lymphocytic thyroiditis and 30% of Graves' disease patients. They are polyclonal and mainly of IgG class with all four subclasses represented. TSH regulates the cell surface expression of TPO and TG altering the transcription of these two proteins, possibly at the gene promoter level. These effects are mimicked by autoantibodies (both blocking and stimulating) in sera of patients with Graves' disease (Collison *et al.*, 1991).

2.3.7 Thyroid stimulating hormone receptor (TSH-R) antibodies

Thyroid stimulating hormone receptor (TSH-R) is the prime autoantigen in Graves' disease and atrophic thyroiditis. It is located on the basal surface of thyroid follicular cells (Boelaert *et al.*, 2005). In Graves' disease, thyroid stimulating antibodies (TSAbs) bind to the receptor and stimulate the thyroid cell to produce excessive amount of thyroid hormones resulting in hyperthyroidism. In patients with atrophic thyroiditis, the major antibody is the TSH to its receptor, thus preventing stimulation of thyroid cell. This results in diminished thyroid hormone output, atrophy of thyroid gland and the clinical state of hypothyroidism (Prabhakar *et al.*, 1997; Boelaert *et al.*, 2005).

2.3.8 Mechanism of thyroid cell injury

Several antibody and cell-mediated mechanisms contribute to thyroid injury in autoimmune thyroid disease. In general, in cases of Hashimoto's thyroiditis, the expression of death receptor CD95 and death receptor ligands CD95L in the thyroid tissue appear to be much higher compared to their normal counterparts. Also the expression of positive effectors of apoptosis, caspase 3 and 8 as well as Bax and Bak appear to be relatively high in thyroiditis samples as compared to controls. This expression pattern supports enhanced apoptosis as the mechanism underlying the loss of thyrocytes in Hashimoto's thyroiditis (Stassi *et al.*, 2002). In Graves' disease, there is highly elevated expression of negative modulators of apoptosis (cFLIP, Bcl-2 and Bcl-XL). This supports the role for apoptosis inhibitory mechanism. Although in both cases there is significant expression of Fas/CD95 and its ligand, only in Hashimoto's thyroiditis, the thyrocytes undergo apoptosis (Prasad *et al.*, 2003). The role of cytokines in the development of autoimmune disorders has also been explained (Stassi *et al.*, 2002). In case of Hashimoto's thyroiditis, a TH1 disease, the cytokine interferon-gamma appears to play a crucial role in the pathology of the disease by enhancing the expression of caspases and thereby sensitizing cells to FAS mediated apoptosis. In contrast in the TH2 mediated Graves' disease, the cytokines IL4 and IL-10 regulate the expression of two anti-apoptotic proteins Bcl-XL and cFLIP, which offers resistance to Fas mediated apoptosis. This proves the necessary modulatory roles played by the TH1 and TH2 cytokines in the development of autoimmune disorders (Prasad *et al.*, 2003).

2.3.8.1 B Cell responses

Thyroglobin (TG) and TPO antibodies occur in very high concentration in patients with Hashimoto's thyroiditis and primary myxedema. These antibodies are less common, but still frequent in Graves' disease, where as TPO rather than TG antibodies are frequent in postpartum thyroiditis (Smallridge, 1996). Both of the antibodies show partial restriction to the IgG4 subclass (McIntosh *et al.*, 1998). TG antibodies usually mediate antibody mediated

cytotoxicity (ADCC), whereas TPO antibodies form terminal complement complexes within the thyroid gland. Cell mediated injury may be necessary for TPO antibodies to gain access to their antigen and become pathogenic (Nilsson *et al.,* 1998).

2.3.8.2 T Cell responses

Both CD4+ and CD8+ T cells occur in thyroid lymphocytic infiltrate with a preponderance of CD4+ cells. There is an increase in activated T cell expressing markers like HLA-DR. Cytokines including IL-2, interferon gamma, tumor necrosis factor, IL-4, IL-6, IL-2, IL-10, IL-12, IL-13, and IL-15 are produced by the lymphocytes with some variation between patients (Weetman, *et al.,* 1997). Thyroid cells express MHC class II and behave as antigen presenting cells (APC). Expression of ICAM-1, LFA-3 and MHC class I by thyrocytes is enhanced by IL-1, tumor necrotic factor and interferon-gamma (Weetman, *et al.,* 1990). This response increases the ability of cytotoxic T cell to mediate lysis.

Humoral immunity exacerbates cell-mediated damage both by direct complement fixation (TPO antibodies) and by ADCC (Chiovato *et al.,* 1993). Complement attack initiated via the classic or alternative pathway, impairs the metabolic function of thyroid cells and induces them to secrete IL-1, IL-6, reactive oxygen metabolites and prostaglandins. All of these enhance the autoimmune process (Simons *et al.,* 1998).

2.4 Biochemical and Hematological Parameters in Immunological Thyroid Diseases

2.4.1 Biochemical parameters in immunological thyroid diseases

Thyroid dysfunction causes remarkable changes in glomerular and tubular functions and electrolyte and water homeostasis. Hypothyroidism is accompanied by a decrease in

glomerular filtration, hyponatremia, and an alteration of the ability for water excretion. Excessive levels of TH generate an increase in glomerular filtration rate (GFR) and renal plasma flow (RPF) (Birewire *et. al.*, 2002). Renal disease in turn leads to significant changes in thyroid function. The association of different types of glomerulopathies with both hyper- and hypofunction of the thyroid has been reported (Hennessey, 1999). Less frequently, tubulointerstitial disease has been associated with functional thyroid disorders. Nephrotic syndrome is accompanied by changes in the concentrations of TH due primarily to loss of protein in urine. Acute kidney injury and chronic kidney disease are accompanied by notable effects on the hypothalamus–pituitary–thyroid axis. The secretion of pituitary thyrotropin (TSH) is impaired in uremia. Contrary to other non-thyroidal chronic disease, in uraemic patients it is not unusual to observe the euthyroid state syndrome with low serum triodothyronine (T_3) (Grymula *et. al.*, 2007).

Thyroid dysfunction causes significant changes in kidney function. Both hypothyroidism and hyperthyroidism affect renal blood flow, GFR, tubular function, electrolytes homeostasis, electrolyte pump functions, and kidney structure (Grymula *et. al.*, 2007). The most common kidney derangements associated to hypothyroidism are: elevation of serum creatinine levels, reduction in GFR and renal plasma flow (RPF), disruption of the capacity to excrete free water and hyponatremia. These alterations may be absent in patients with central hypothyroidism due to the fact that this kind of thyroid hypofunction is often accompanied by other pituitary hormone deficiencies that might affect directly or indirectly the kidney function (Birewire *et. al.*, 2002). Thyroid dysfunction is known to influence serum creatinine levels. Decreased creatinine clearance and eventually increased creatinine release by muscle cells seem to be responsible for elevated serum creatinine levels observed in patients with hypothyroidism (Grymula *et. al.*, 2007; Birewire *et. al.*, 2002). In contrast, decreased serum creatinine levels may be encountered in patients with hyperthyroidism. Creatine is one of the

several different types of amino acids that animals produce. In the human body, this building block of protein is synthesized within the pancreas, liver and kidney. In human body around 95 percent of your body's creatine is stored within skeletal muscles, where it is converted into creatine phosphate or phosphocreatine and used as a fuel source for muscular activity (Foster, 1999). During high-intensity exercises, such as power-lifting and high jumping, phosphocreatine turns into adenosine triphosphate (ATP), one of the body's major energy sources. The baseline creatine level is determined by age, gender, and ethnic background, lean body mass and physical activity (Birewire *et. al.,* 2002). Medical research has revealed, however, that creatine levels are also inversely related with activity of the thyroid hormone. A study from Indian researchers found that patients with hyperthyroidism, or high thyroid activity, produced significantly lower amounts of creatine than normal (Foster, 1999). The reverse held true for patients with hypothyroidism, who produce significantly higher levels of creatinine than normal (Birewire *et. al.,* 2002).

Primary hypothyroidism is associated with a reversible elevation of serum creatinine in both adults and children. This increase is observed in more than half (~55%) of adults with hypothyroidism. Moreover, some authors have reported an elevation of serum creatinine associated with subclinical hypothyroidism. Primary hypothyroidism is associated with a reduction of GFR and RPF that are normalized following levothyroxine administration (Jayagopal *et. al.,* 2003). Similarly, normalization of circulating TH concentrations with replacement therapy in hypothyroid patients with chronic kidney disease (CKD) can significantly improve GFR (Birewire *et. al.,* 2002). However, it has recently been reported that kidney function recovers slowly in hypothyroid children, and sometimes partially, after the introduction of replacement with levothyroxine (Jayagopal *et. al.,* 2003). The long-term clinical implications of these findings are unknown. Hypothyroidism-associated kidney

dysfunction seems to be more related with the decline in thyroid hormone levels rather than with thyroid autoimmunity.

Among the mechanisms involved in hypothyroidism-associated kidney derangements are direct effects of TH on the cardiovascular system (increased peripheral resistance and reduction of myocardial contractility and stroke volume) and metabolism (hyperlipidemia), and indirect effects through paracrine or endocrine mediators, such as insulin-like growth factor type 1 (IGF-1) and vascular endothelial growth factor (Birewire *et. al.*, 2002).

Thyrotoxicosis is characterized by an increase in RPF and GFR resulting in a reduction of serum creatinine levels (Grymula *et. al.*, 2007). These changes are normalized after the control of thyroid function with appropriate treatment. Hyperthyroidism may be linked to a decrease in total body water and exchangeable potassium (K^+). By contrast, the amount of exchangeable sodium (Na^+) tends to increase. However, serum concentrations of Na^+, K^+, and chloride (Cl^-) are normal (Birewire *et. al.*, 2002). These alterations are typical of endogenous hyperthyroidism and exogenous thyrotoxicosis. However, central hyperthyroidism may not be accompanied by these changes when it is associated with other pituitary disorders (Grymula *et. al.*, 2007).

2.4.2 Hematological parameters in immunological thyroid diseases

Thyroid hormones (THs) play an important physiological role in humans. Including regulation of human haematopoiesis in the bone marrow (Golde, 1977). The association of thyroid disorders and abnormalities in hematological parameters is well known. Earlier studies showed that Graves' disease is associated with anemia (Fein, 1979). A decreased number of red blood cells (RBCs) were seen in the peripheral blood (PB) of patients after thyroidectomy (Horton *et al.*, 1976). Hypothyroidism can cause certain forms of anemia on

the one hand or hyperproliferation of immature erythroid progenitors on the other hand. Usually macrocytic hypochromic anemia of moderate severity is seen in patients with hypothyroidism (Horton *et al.,* 1976). In contrast, anemia is not frequently observed in patients with hyperthyroidism, whereas erythrocytosis is fairly common (Fein & Rivlin, 1979; Corrocher *et al.,* 1981). It has been found that all hematological parameters return to normal when euthyroid state is achieved (Perlman *et al.,* 1983). As far as white blood cells and thrombocytes are concerned, a slightly depressed total leucocyte count, neutropaenia and thrombocytopaenia have been observed in hypothyroid patients (Lima *et al.,* 2006). Furthermore, elevated, normal or slightly depressed total leukocyte counts have been found in hyperthyroid patients, with only a relative decrease in the number of neutrophils and a relative increase in the number of eosinophils and mononuclear cells (MNCs). Nevertheless, hyperplasia of all myeloid cell lines in hyperthyroidism and their hypoplasia in hypothyroidism have been reported by earlier studies (Axelrod and Bergman, 1951).

With regard to lymphocytes, triiodothyronine (T_3) has been shown to be a prerequisite for normal B cell production in the bone marrow through its regulation of pro-B cell proliferation (Foster *et al.,* 1999; Arpin *et al.,* 2000; Grymula *et al.,* 2007). The observations previous studies confirmed the association between thyroid gland dysfunction and haematopoiesis. Previously published studies suggested that there is an essential relationship between the hypothyroid state and low levels of iron, vitamin B_{12} and folic acid in the human body (Horton *et al.,* 1976; Hines *et al.,* 1968). Furthermore, it has been postulated that the influence of THs on haematopoiesis involves an increased production of erythropoietin or haematopoietic factors by non-erythroid cells (Dainak *et al.,* 1986; Fandrey *et al.,* 1994). However, a growing number of studies have demonstrated a direct role of THs in normal human and animal erythropoiesis (Malgor *et al.,* 1975; Golde *et al.,* 1977; Schroeder *et al.,* 1992; Perrin *et al.,* 1997; Leberbauer *et al.,* 2005).

All forms of thyroid autoimmunity typically start with T and B cells which infiltrate the thyroid gland in equal numbers (McIntosh *et al.,* 1998). These white blood cells are the primary infection-fighting immune cells. T cells identify invasive molecules, such as viral proteins, and help B cells to produce antibodies that specifically attack these invaders (Wu *et al.,* 1994). In cases of autoimmunity, T cells are tricked into classifying molecules on the body's own cells as invaders. In such cases, B cells then produce antibodies, called autoantibodies, which attack self cells. In most cases of thyroid autoimmunity, the autoantibodies launch an attack on a thyroid protein called thyroid peroxidase which appears to destroy thyroid cells (Collison *et al.,* 1991).

Thyroid hormones play critical roles in differentiation, growth and metabolism (Yen, 2001). Their release from the thyroid gland involves a fine tuned regulated mechanism that includes structures of the central nervous system. Low circulating levels of thyroid hormones are sensed in the hypothalamus, which responds by releasing thyrotropin releasing hormone (TRH). The TRH stimulates the pituitary to produce thyrotropin or thyroid stimulating hormone (TSH), which, in turn, stimulates the thyroid gland to produce thyroid hormone until levels in the blood return to normal. These hormones are then transported by the blood to many different target tissues where they regulate the transcription of genes controlling cell metabolism. This system is down-regulated by thyroid hormones (T3 and T4), which provide a negative feedback control on both the hypothalamus and the pituitary gland, thus controlling the release of TRH and TSH, respectively (Yen, 2001).

With respect to their relation with the immune system, thyroid hormone participation in primary and secondary lymphopoiesis has been described (Fabris *et al.,* 1995; Foster *et al.,* 2000). However, some evidence raised from analysis of lymphocyte development and function in mice with genetic defects in the expression of thyroid hormones or their receptors,

suggested that these hormones are not necessarily required for the development of a normal immune response. In fact, no alteration in humoral or cell-mediated immunity was found in dwarf mice with genetic defects in genes encoding for several anabolic hormones (including thyroid hormones) or in thyroid hormone deficient *hyt/hyt* knockout (Foster *et al.*, 2000). Other studies have hypothesized that thyroid hormones are involved in immune system homeostasis maintenance in response to environmental changes or stress-mediated immunosuppression (Davis, 1998; Dorshkind, *et al.*, 2000).

On the other hand, the presence of functional receptors for hypothalamus-pituitary-thyroid axis (HPT) hormones on lymphocytes as well as the frequent immune alterations observed during physiological or pathological fluctuations of thyroid hormones strengthens the interactions between the HPT axis hormones and the immune system (Fabris *et al.*, 1995). The presence of triiodothyronine (T_3) in lymphocytes, mast cells, monocytes, macrophage and granulocytes from rat peritoneal fluid and blood, and in thymic lymphocytes has been demonstrated (Csaba *et al.*, 2004). This extrathyroidal source of T_3 is regulated by TSH and is required for maintaining cell proliferation and the normal status in the immune system (Csaba *et al.*, 2004; T Csaba *et al.*, 2009). Thus, in order to analyze the role that thyroid hormones exert on immunity in individuals with different thyroid status, but without autoimmune pathology, it is important to clarify the effect of thyroid axis on immune function.

In conclusion, one could generalize that low levels of thyroid hormones *in vivo* lead to a decrease of the immune function, while the opposite occurs when circulating levels of these hormones are high. Despite the proven direct action of thyroid hormones on immune cells, the possible involvement of other endocrine factors regulated by thyroid status cannot be ruled out and hence it is important to clarify the effect of thyroid axis on immune function as was done in this thesis research.

2.5 Conceptual Framework

Based on the literature review, various concepts have been identified and put in the conceptual framework in Figure 1 showing immunological thyroid diseases and the associated diagnostic factors.

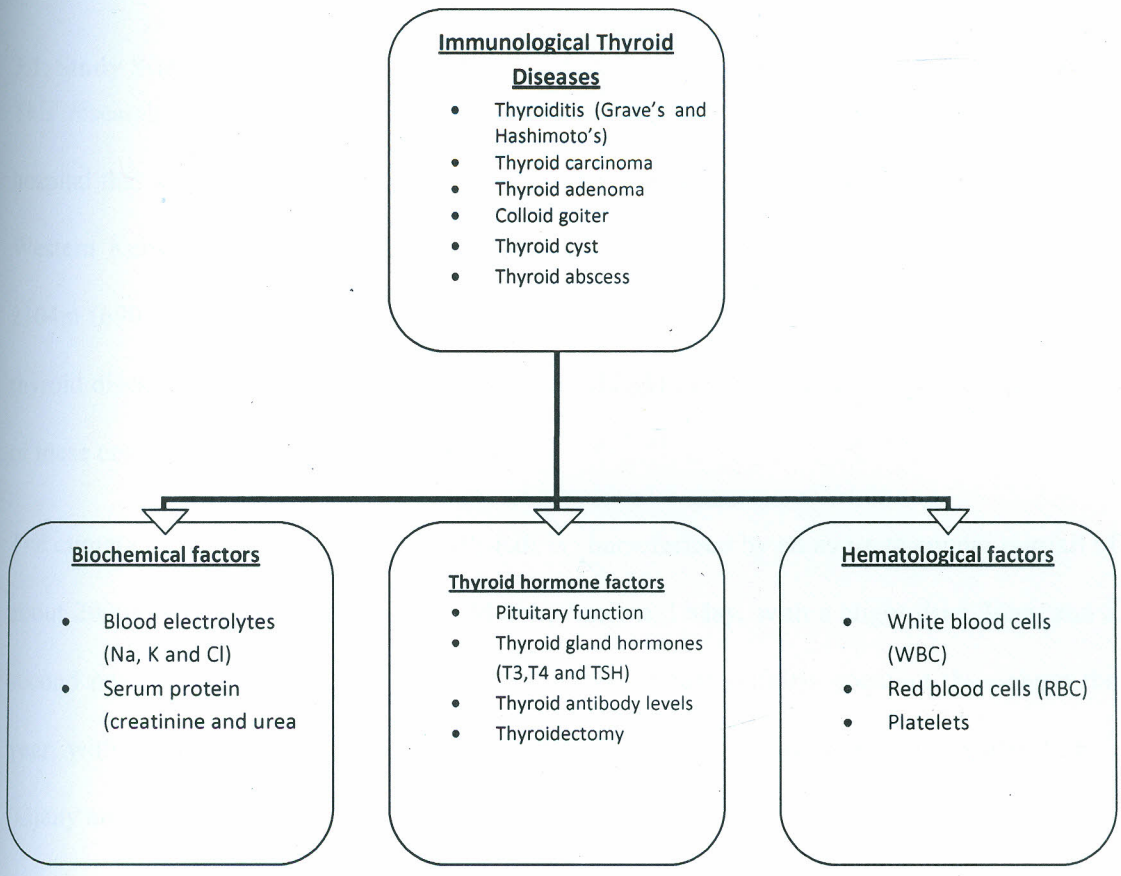

Figure 1: Conceptual framework showing immunological thyroid diseases and the associated diagnostic factors

CHAPTER THREE

3.0 METHODOLOGY

In this chapter, the study area, research design, study population and sampling procedures are given. In addition the chapter also outlines the data collection procedure, data analysis and presentation.

3.1. Study Site

This research was carried out at Moi Teaching and Referral Hospital-Eldoret. This is a hospital that serves clients from all over North-Rift, parts of western and Nyanza province in Western Kenya. Moi Teaching and Referral Hospital (MTRH) is situated at an altitude of 2104m (6903 feet) and 0°31'N 35°17'E of the prime meridian (Appendix I). A number of thyroid disease patients are seeking treatment at MTRH in which the etiology and prevalence of these conditions have not been reported.

The climate of western Kenya and North-Rift is characterized by an average annual rainfall of about 2000mm. Rainfall is heaviest in March, April and May, with a slight drier June, and a second peak roughly in August to September. Temperature is fairly constant throughout the year, with mean daily minimums of about 11^0C and a mean daily maximum of about 26^0C (Ojany and Ogendo, 1988).

3.2 Study Design

This was a retrospective survey study in which all patients with thyroid pathologies but not on immunosuppressant drugs, who underwent thyroidectomy at the MTRH between 2008 and 2011 were included. Clinical data on thyroxine hormone (FT4), triiodothyronine hormone (FT3), TSH hormones' profile, urea, creatinine, electrolytes, Hb, WBCs, RBCs, platelets and histology pathology reports was obtained from the hospital record files for purposes of documenting the disease trends over a period of four years. Stored tissues were retrieved and

processed for immunohistochemical studies by detection of the thyroid autoantibodies directed against thyroid antigen.

3.3 Study Population and Sample Size Determination

The ear, nose and throat (ENT) department of MTRH-Eldoret saw at least 3 thyroid patients every week, 12 per month and 144 per year. This was not designed but a pure coincidence. This information was obtained from the hospital medical records. Therefore for four years (2008-2011) the number of patients seen at MTRH was 576. The prevalence of autoimmune thyroiditis in Kenya is approximately 2% (0.02) (CureResearch, 2005), but this is believed to be extrapolated. An effect size of 0.03 was used and the level of significance was taken to be 1.96. Power analysis for this study was determined which showed that the power of the study was 88.89% (89%) at 95% confidence interval, which means that the results obtained are reliable and can be used for generalization (Russell, 2001).The sample size at 95% confidence interval required to estimate the prevalence of autoimmune thyroid disease was estimated using the formula below (Cochran and William, 1963; Russell, 2001).

$$N = (Z_{crit})^2 * P (1-P) / E^2$$

Whereas; P = Prevalence (0.02).

N = Sample size,

E= effect size (0.03).

Z_{crit} = level of statistical significance (1.96).

$N = (1.96)^2\ 0.02\ (0.98) / 0.03^2$

N = 83.6615 or 84.

The minimum sample size that was therefore arrived at is 83.6615 or 84

3.4. Ethical Considerations

Ethical clearance for the present study was obtained from the Institutional Review Ethics Committee (IREC) at the MTRH (see appendix II & III). Authorization to carry out the research was sought from the Director, School of Graduate Studies (SGS) Maseno University. Data collected was regarded as confidential.

3.5 Study Procedures

3.5.1 Clinical and biochemical examination

Triiodothyronine hormone (T3), thyroxine hormone (T4) and thyroid stimulating hormone (TSH) levels were measured using enzyme-linked immunosorbent assay (ELISA) for quantitative determination of hormones concentration in human serum/plasma (Helenius, 1986). Whole blood samples were collected through venipuncture, centrifuged at 3000 rpm and then frozen at -20^{0}c for storage if to be measured later. This was done before the patients were included in the study. Serum urea, creatinine and electrolytes (sodium, potassium and chloride) were measured before thyroidectomy. Serum T3, T4 and TSH and hematological profile were also measured preoperatively.

3.5.2 Histology

All thyroid specimen collected during thyroidectomy were then transferred to histology department of MTRH for histological processing. The tissues were formalin fixed (see appendix IV), paraffin embedded sections sliced using a microtome and slices were mounted on slides (see appendix V). The slices were then stained with Haematoxilin and Eosin (H&E) (see appendix VI). These sections were diagnosed and reported by a pathologist. Information collected included patients age, sex and diagnosis made.

During the study period, the tissue were resectioned and stained again for the purpose of validation. As the examination was going on, the slides were photographed at the same magnification using a Kodak camera mounted on the same microscope. Also histological

feature of each thyroid pathology examined were recorded. Each slide was then dipped into xylene after every examination to remove the oil immersion from its surface and stored in a slide box for future references.

3.5.3 Immunohistochemistry

About 4-40 micrometers (μm) thick of formalin and paraffin embedded sections were sliced using a microtome and slices were mounted on slides. The slides were then taken for immunohistochemistry processing in which the antigen was localized in cells of the thyroid tissue section exploiting the principle of antibody specificity to antigens in biological tissues (see appendix VII). Immunohistochemical staining was used to diagnose abnormal cells such as those found in thyroid cancer and Hashimoto's thyroditis. Visualization of antibody - antigen reaction was accomplished through an antibody (IgG) conjugated to FITC anti-human globulin that catalyzed a color (apple green) producing reaction which indicated a positive reaction when viewed under fluorescence microscope (see appendix VIII) (Baloch *et al.*, 2003). As the examination was going on, the slides were photographed at the same magnification using a Kodak camera mounted on the same microscope. However, the observations made were recorded in terms of absence or presence of the autoantibody. Also histological feature of each thyroid pathology examined were recorded. Each slide was then dipped into xylene after every examination to remove the oil immersion from its surface and stored in a slide box for future references.

3.5.4 Determination of the prevalence of immunological thyroid diseases

Patients with thyroid pathologies who were not on any immunosuppressant drugs and underwent thyroidectomy at the MTRH between 2008 and 2011 were recruited. Prevalence was calculated as the proportion of patients with thyroid disease divided by the total number of patients examined for immunological thyroid disease expressed as a percentage. The

corresponding 95% confidence limits were calculated. The number of patients with a particular thyroid disease and the prevalence value were tabulated. The prevalence was computed, stratified by gender and for combined groups.

3.5.5 Determination of the serum levels of TSH, T3, T4 (hormones), biochemical and hematological profiles

3.5.5.1 Determination of the serum levels of TSH hormones

In the determination of serum levels of TSH hormones, the Biocheck TSH ELISA was used which was based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody was for solid phase immobilization (microtitre wells) and goat anti-TSH antibody was in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the true antibodies, resulting in the TSH molecule being sandwiched between the solid phase and enzyme-linked antibodies. After 60 minutes or overnight incubation at room temperature, the solid phase was washed with buffer (working wash solution) to remove unbound labeled antibodies. A solution of 3, 3', 5, 5'-tetramethylbenzidine (substrate (TMB)) was added and incubated for 20 minutes at room temperature resulting in the development of a blue color. The color development was stopped with the addition of 3N HCL, and the resulting yellow color was measured spectrophotometrically at 450nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

3.5.5.2 Determination of the serum levels of T3 hormones

In the determination of serum levels of T3 hormones, the Biocheck T3 enzyme linked-immunosorbent assay (ELISA) was used. In this, goat anti-mouse IgG was coated on a

microtitre wells. A measured amount of patient serum, a certain amount of monoclonal anti-T3 antibody and a constant amount of T3 conjugated with horseradish peroxidase were added to the microtitre wells. After 60 minutes incubation at room temperature, the wells were washed 5 times with buffer (1X PBS) to remove unbound T3 conjugate. A solution of H_2O_2/ TMB was then added and incubated for 20 minutes at room temperature resulting in the development of a blue color. The color development was stopped with the addition of 1N HCl and the absorbance was measured spectrophotometrically at 450nm wave length. The intensity of the color formed is proportional to the amount of enzyme present and was inversely related to the amount of unlabelled T3 standards assayed in the same way. The concentration of T3 in the unknown sample is then calculated.

3.5.5.3 Determination of the serum levels of T4 hormones

In the determination of serum levels of T4 hormones, the Biocheck T4 enzyme linked-immunosorbent assay (ELISA) was used. In this, anti-T4 antibody was coated on a microtitre wells. A measured amount of patient serum, a constant amount of T4 conjugated with horseradish peroxidase was added to the microtitre wells and incubated. After 60 minutes incubation at room temperature, the wells were washed 5 times with PBS to remove unbound T4 conjugate. A solution of 3, 3', 5, 5'- tetramethylbenzidine (substrate (TMB)) was then added and incubated for 20 minutes at room temperature resulting in the development of a blue color. The color development was stopped with the addition of 1N HCl and the absorbance was measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabelled T4 standards assayed in the same way. The concentration of T4 in the unknown sample was then quantified.

3.5.5.4 Determination of the serum levels of biochemical parameter (electrolytes, urea and creatinine)

In the laboratory facility, serum was aliquoted and stored at -70°C until enzymatic determinations. The following parameters were analyzed using the dry method in a Vitros 950 system (Johnson & Johnson, Rochester, NY): electrolytes (Na^+, K^+ and Cl^-), urea and creatine.

3.5.5.5 Determination of the hematological profiles

Hemoglobin concentration was determined by a colorimetric method with the addition of a sample centrifugation (1,600 × *g*, 5 min) before reading (Campbell, 2004a). Erythrocytes (red blood cells) and thrombocytes were counted simultaneously in a Neubauer chamber using the modified Dacie's fluid with the addition of brilliant blue cresyl (Campbell, 2004b). Mean corpuscular volume and mean concentration of corpuscular hemoglobin were calculated according to Jain method (Jain, 1993).

The leucocyte concentration [white blood cells (WBC)] was obtained through the counting of these cells in a Neubauer chamber using heparinized blood. Because heparin causes leukocyte destruction in ostrich blood (Green and Blue-McLendon, 2000), the WBC concentration was also indirectly determined by a method described previously (Tavares-Dias and Moraes, 2006). Briefly, leucocytes and erythrocytes were counted separately along the smear up to a total of 2,000 cells; a ratio was determined and the WBC concentration was indirectly calculated using the red blood cell count performed as described above. The differential count of leukocytes was made in blood smears stained with Diff-Quick.

3.6 Data Analysis and Presentation

Data analysis was done using statistical data analysis package called STATA, Version SE/10 (College Station, Texas, USA). Categorical variables were summarized as frequencies (percentages) while the continuous variables were summarized as median (quartiles) since they had skewed distributions. Association between the primary explanatory variables and the outcome was assessed using logistic regression model and the associations given in terms of odds ratios and the corresponding 95% confidence limits. Median test was used to compare the distributions (medians) of the different groups. Age was categorized at an interval of 10 years. These limits were clinically acceptable limits. The comparisons of the proportions was done using the Wald test of proportions, while the test of association between the categorical variables was conducted using the Pearson's Chi-square test and the Fisher's exact test. Comparison of the distributions of the outcomes between two groups was conducted using Wilcoxon rank-sum test. Data presentation was done using bar graphs, tables and pie charts.

CHAPTER FOUR

4.0 RESULTS

There were 388 subjects aged between 14 and 89 years who were eligible for analysis and were categorized as having been diagnosed with thyroid gland disorders, autoimmune thyroid disease or not.

4.1 Determination of the prevalence and histological patterns of immunological thyroid diseases in patients undergoing thyroidectomy at the Moi Teaching and Referral Hospital (MTRH) in western Kenya

The frequencies and proportions are shown in Table 1a and 1b. Histological pictures were used to categorize the conditions as thyroiditis, thyroid carcinoma, thyroid adenoma, colloid goitre, thyroid cysts or thyroid abscess see Plates 1a, 1b, 1c, 1d, and 1e.

Table 1: Frequency of histological categories of thyroid gland disorders

Histological categories of thyroid gland disorders	Frequency n (%)
Thyroiditis	24 (7.2)
Thyroid Carcinoma	18 (4.6)
Thyroid Adenoma	51 (13.1)
Colloid Goitre	286 (73.7)
Thyroid Cyst	8 (2.1)
Thyroid Abscess	1 (0.3)
Total	**388 (100)**

There were 24 (7.2%; 95% confidence limits (CL): 4.0-9.1) subjects who had the autoimmune thyroid disease with subjects aged above the median of 41 (IQR: 32-50) having a non significant higher rate (p-value=0.654) of autoimmune disease (6.4%; 96% CL: 3.0-10.0)

compared to those aged below the median (5.4%; 95% CL: 2.0-8.9). This comparison of the proportions was done using the Wald test of proportion. Table 1b shows autoimmune thyroid diseases (thyroiditis types) and their percentages.

Table 2: Patterns of autoimmune thyroid diseases

Autoimmune thyroid diseases (thyroiditis)	Frequency n (%)
Granulomatous thyroiditis	1 (4.17)
Grave's thyroiditis	4 (16.67)
Hashimoto thyroiditis	18 (75.00)
Unspecified Thyroiditis	1 (4.17)
Total	**24 (100)**

The samples were mainly made up of females 368 (95%). There was no case of autoimmune thyroid disease that was reported among the male patients. From table 1a, colloid goiter represented the largest proportion of the histological diseases. Thyroid abscess represented a very small proportion. Plates 1a- shows some of the images of thyroid histology taken during the study and their description.

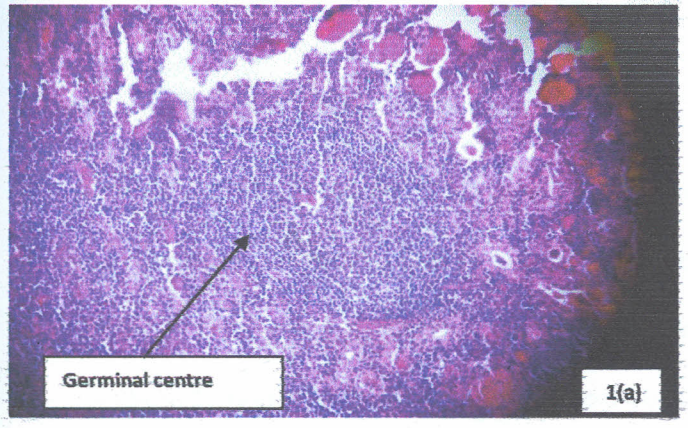

Plate (1a) Hashimoto thyroiditis: The tissues shows late stage Hashimoto's thyroiditis with thyroid architecture distorted by fibrosis with lymphoid follicles containing reactive germinal

centre (lymphocytes, plasma cells and occasional multinuclear giant cells) under x40 magnification.

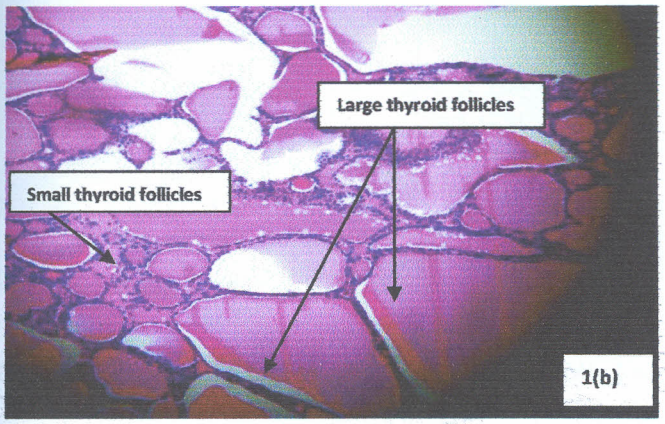

Plate (1b) Colloid goiter: shows colloid goiter with large and small follicles filled with colloid that appears quiescent. Abundant colloid is seen in the hugely distended follicles when viewed under x40 magnification.

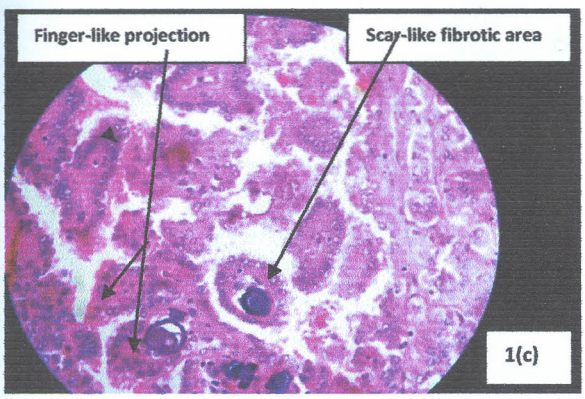

Plate (1c) papillary carcinoma: This tissue section shows scar-like fibrotic area which may intercept neoplastic glands. The fibrous brands may extend outward and divide the tumor into large clusters. Finger-like projections lined by neoplastic cells when viewed under x40 magnification. In normal condition, thyroid gland contains numerous follicles, composed of epithelial follicle cells and colloid. Also, between follicles are clear parafollicular cells, which produce calcitonin (hormone for calcium balance).

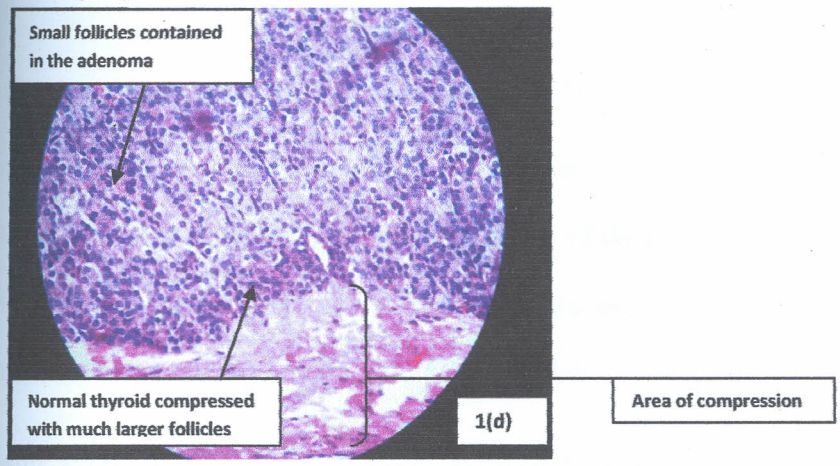

Plate (1d) ***Follicular adenoma:*** shows small follicles contained in the adenoma. The normal thyroid has been compressed with much larger follicles at the bottom than those within the adenoma. There is no invasion of the capsule when viewed under x40 magnification.

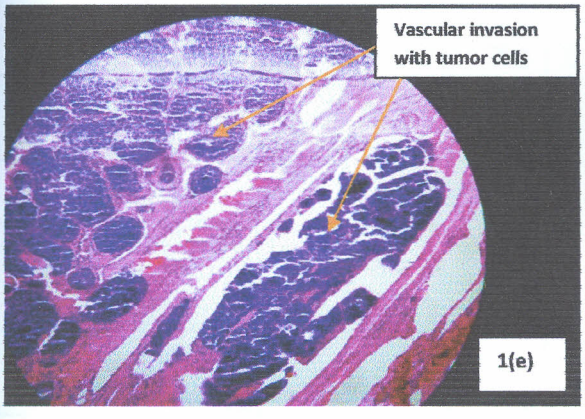

Plate (1e) Follicular carcinoma: The tissue section show vascular invasion in follicular carcinoma with tumor cells invading a capsular vessel. The vascular invasion seen here is evidence for malignancy and hence there is possibility of metastasizing to other tissues. The features of papillary carcinoma are lacking, so this is a follicular carcinoma composed of cells that are not highly pleomorphic or hyperchromatic. It can be difficult to tell a follicular carcinoma from an adenoma by histologic appearance alone, and the term "follicular neoplasm" may be utilized. Follicular carcinoma, the second most common thyroid malignancy, tends to be indolent.

The histological disease patterns stratified by age categories and by sex are given in Tables 2 and 3, respectively. From the results, colloid goitre represented the greatest proportion across all the age categories. Similarly, the proportions of the patients suffering from the individual histological diseases increased across the age groups. The same picture was apparent when the results were stratified by sex, see Table 3 and 4.

Table 3: Distribution of immunological thyroid diseases by age categories

	Histological categories of thyroid disorders						
Age	**Thyroiditis**	**Thyroid Carcinoma**	**Thyroid Adenoma**	**Colloid Goitre**	**Thyroid Cyst**	**Thyroid Abscess**	**Total**
<20	0 (0.0%)	1 (14.3%)	0 (0.0%)	5 (71.4%)	1 (14.3%)	0 (0.0%)	7 (100%)
20-29	3 (7.14%)	0 (0.0%)	7 (16.7%)	32 (76.2%)	0 (0.0%)	0 (0.0%)	42 (100%)
30-39	6 (5.1%)	2 (1.7%)	17 (14.5%)	91 (77.8%)	1 (0.9%)	0 (0.0%)	117 (100%)
40-49	8 (7.8%)	8 (7.8%)	16 (15.7%)	69 (67.7%)	1 (1.0%)	0 (0.0%)	102 (100%)
50-59	2 (4.1%)	1 (2.0%)	3 (6.1%)	41 (83.7%)	2 (4.1%)	0 (0.0%)	49 (100%)
60-69	2 (8.7%)	0 (0.0%)	4 (17.4%)	16 (69.6%)	0 (0.0%)	1 (4.35%)	23 (100%)
Above 70	1 (3.5%)	5 (17.2%)	2 (6.9%)	20 (69.0%)	1 (3.5%)	0 (0.0%)	19 (100%)
Adults	2 (10.5%)	1 (5.3%)	2 (10.5%)	12 (63.2%)	2 (10.5%)	0 (0.0%)	19 (100%)
Total	**24 (6.2%)**	**18 (4.6%)**	**51 (13.1%)**	**286 (73.7%)**	**8 (2.1%)**	**1 (0.3%)**	**388 (100%)**

Table 42: Distribution of immunological thyroid diseases by sex

	Histological categories of thyroid disorders						
SEX	**Thyroiditis**	**Thyroid Carcinoma**	**Thyroid Adenoma**	**Colloid Goitre**	**Thyroid Cyst**	**Thyroid Abscess**	**Total**
Female	24 (6.5%)	16 (4.4%)	49 (13.3%)	271 (73.6%)	7 (1.9%)	1 (0.3%)	368 (100%)
Male	0 (0.0%)	2 (10.0%)	2 (10.0%)	15 (75.0%)	1 (5.0%)	0 (0.0%)	20 (100%)
Total	24 (6.2%)	8 (4.6%)	51 (13.1%)	286 (73.7%)	8 (2.1%)	1 (0.3)	388 (100%)

The median age of the subjects was 40 (33-50). Stratified by gender, there was no significant difference with regards to age distribution: male 52 (37-73) and female 40 (33-50) with p-value=0.154.

4.2 Determination of the Association between Pituitary, Thyroid Hormones and Thyroid Autoantibody Levels in Immunological Thyroid Diseases Patients

Of the 388 cases examined, the proportion of the subjects with thyroid auto antibodies present was 175 (45%, 95% CL: 40-50). Out of these, 175 subjects with auto antibodies present, 11 (6.3%; 95% CL: 3.2-11.0) had autoimmune thyroid disease. This represents 46% (95% CL: 25.6-67.2) among those subjects suffering from autoimmune thyroid disease. Table 4 shows results for the subjects who had antibody present and those that had antibody absent and their statistical tests for association with thyroid hormones. The median test showed that there was only statistically significant difference in T_3 hormone. Thus having higher levels of T_3 hormone was significantly associated with development of auto antibody (**p=0.009**). The rest of the hormones did not show any significant association with the development of auto antibodies though their levels were elevated compared to those without auto antibodies.

Table 4: Association between thyroid hormones and thyroid autoantibody among the immunological patients

			Autoimmune thyroid disease				
	Overall		Ab Present		Ab Absent		Test for difference
variable	sample size (n)	Median (Q1-Q3)	sample size (n)	Median (Q1-Q3)	sample size (n)	median (Q1-Q3)	p-values
age	166	41(32-50)	11	40(32-54)	155	41(32-50)	0.787
TSH	144	1.53(0.7-2.6)	11	2.6(1.09-3.6)	133	1.5(0.7-2.6)	0.323
T3	136	1.56(0.9-3.1)	10	2.6(1.7-3.1)	126	1.4(0.9-3.2)	**0.009**
T4	139	1.08(0.7-1.9)	10	1.4(1.0-2.0)	129	1.01 (0.7-1.8)	0.181

Similarly, the Fisher's exact test for the association between the immunohistochemistry test results and the presence or absence of the thyroid diseases showed that there existed a statistically significant relationship between the two with a p-value = 0.001, Table 5.

Table 5: Test of association between thyroid autoantibodies and immunological thyroid diseases

	Autoantibodies	Autoimmune thyroid diseases: Non-immunological	Autoimmune thyroid diseases: Immunological	Total
Immunohistochemistry test results	Ab (+) present	175	0	175
	Ab (-) absent	0	213	213
	Total	**175**	**213**	**388**

Fisher's exact test of association **p-value=0.001.**

Plates 2(a) and 2(b) show thyroid sections with autoantibodies staining bright apple green fluorescence (arrows) when incubated with anti-human globulin IgG conjugated to fluorescein isothiocyanate (FITC). Patterns of staining can vary from nuclear and/or

cytoplasmic staining and can be exhibited depending on the types and relative amounts of autoantibodies present in the tissue section. Below are thyroid tissues from a patient with colloid goiter showing dense deposits of IgG along the basement membrane of follicular cells under high power microscopy (fluorescent microscope) at X 40 magnification.

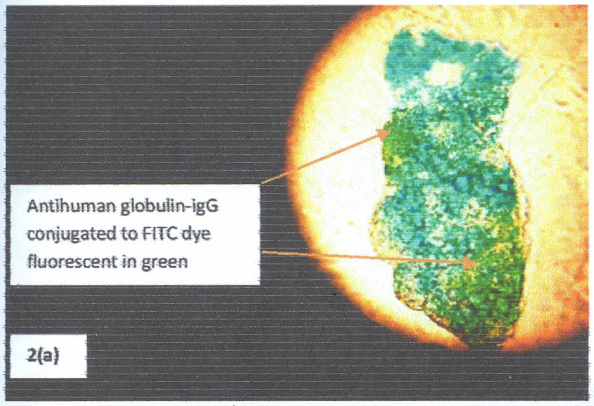

Plate (2a) Positive slide for autoantibodies

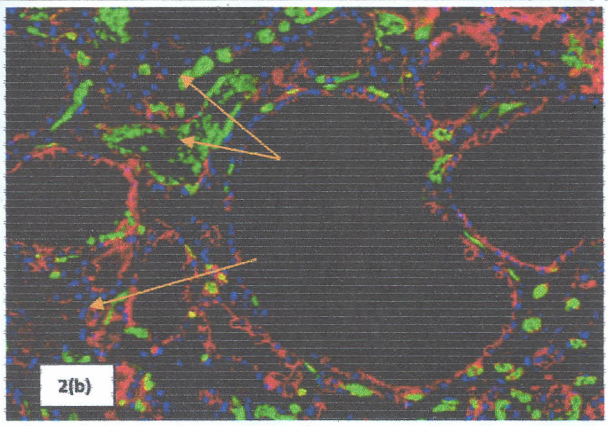

Plate (2b) Positive slide for autoantibodies showing the location of autoantibodies within the thyroid gland.

The proportions of colloid goiter among those patients who did not have immunological thyroid disease (not immunological) was higher than in the patients in the immunological group while the proportion of subjects suffering from thyroid adenoma was higher in the immunological group (group that were positive when tested with anti-human globulin IgG = 213 subjects) compared to the non-immunological group (group that were negative when tested with anti-human globulin IgG = 175 subjects). However, the rest of the histological

diseases showed no visible difference in their proportions between the two groups except for the subject suffering from thyroid abscess who was non-immunological, see Figure 2.

Figure 2: Proportion of histological thyroid diseases stratified by immunological and non-immunological

Figure 3 shows the thyroid stimulating hormone (TSH) levels of the subjects suffering from autoimmune thyroid disease (Yes) and those not suffering from the autoimmune thyroid disease (No) stratified by age groups. The TSH serum levels were high for those subjects suffering from the autoimmune thyroid disease across all the age groups except for those aged 40-49 and 50-59 years. However, the differences were not statistically significant at 5% level of significance except for those aged 30-39 years (**p =0.023**). Comparison of the distributions of the outcomes between the two groups show those subjects suffering from autoimmune thyroid disease (yes) and those not suffering from autoimmune thyroid disease (No) in the figure below was conducted using Wilcoxon rank-sum test.

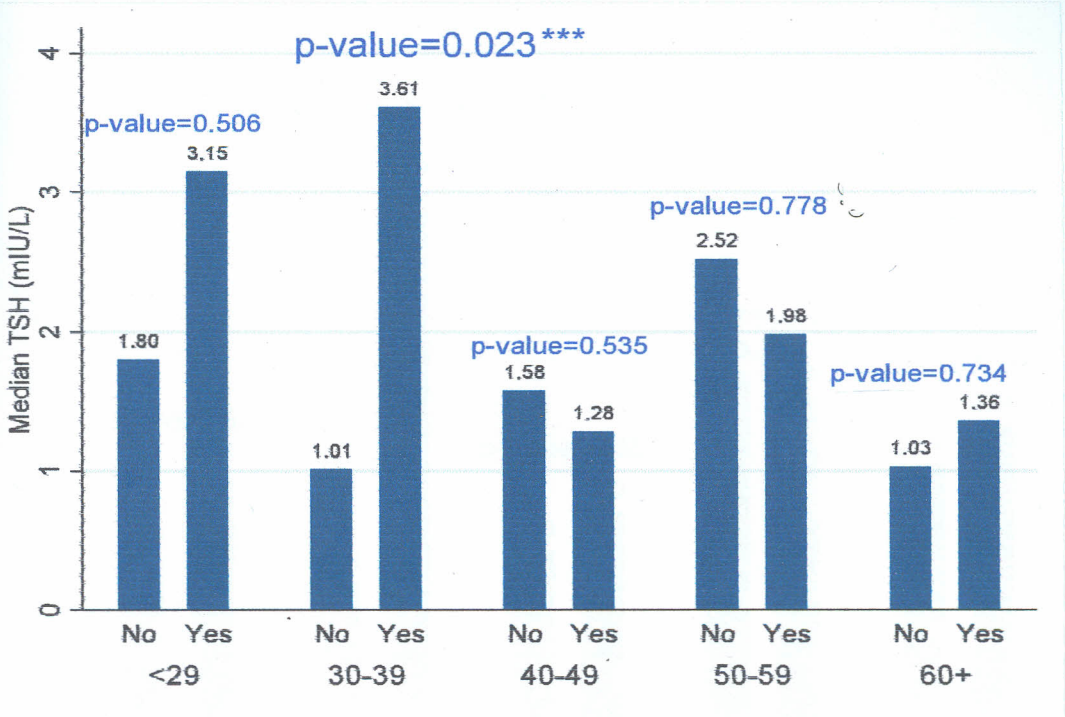

Figure 3: Thyroid stimulating hormone (TSH) levels among the immunological thyroid disease patients

The Triiodothyronine (T_3) levels were high for those subjects suffering from the autoimmune thyroid disease across all the age groups except for those aged 50-59 years. However, the differences were not statistically significant at 5% level of significance except for those aged 30-39 years (**p=0.04**). Comparison of the distributions of the outcomes between the two groups that show those subjects suffering from autoimmune thyroid disease (yes) and those not suffering from autoimmune thyroid disease (No) in figure 4 was conducted using Wilcoxon rank-sum test.

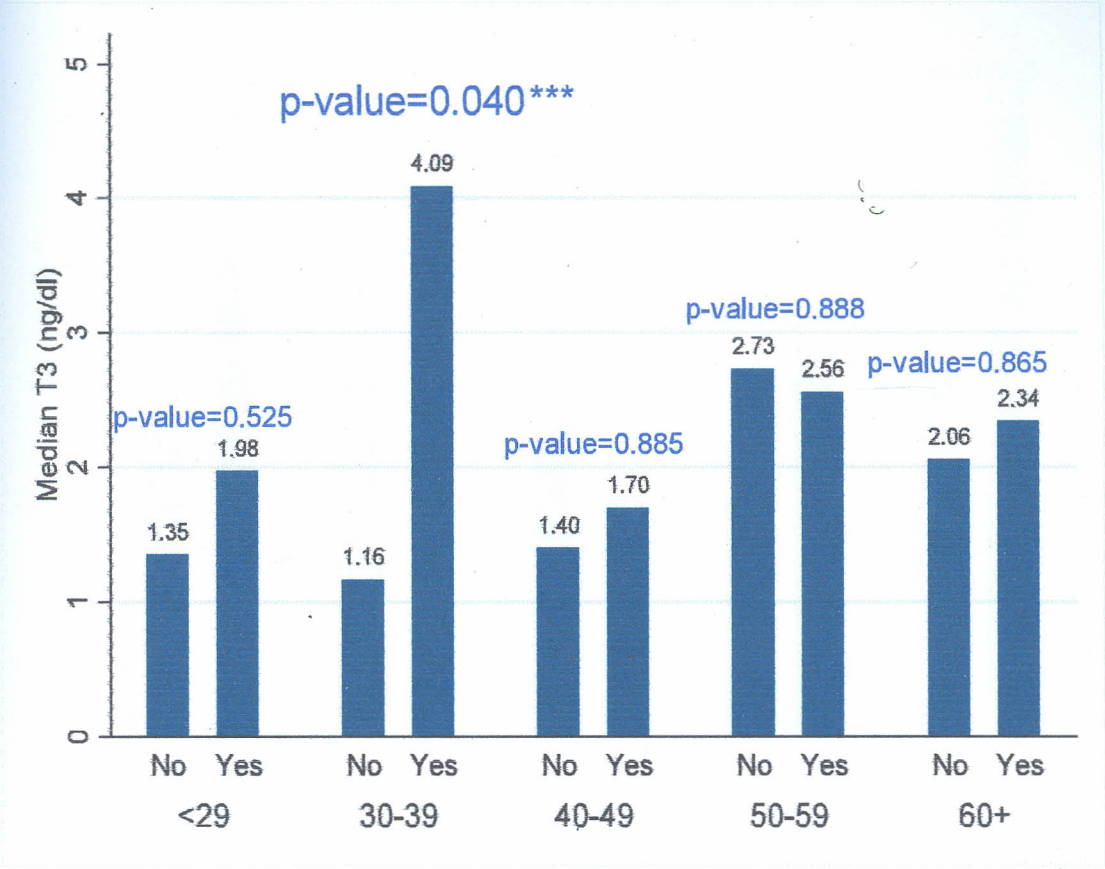

Figure 4: Triiodothyronine (T_3) levels among the immunological thyroid disease patients.

The Thyroxine hormone (T_4) levels were high for those subjects suffering from the autoimmune thyroid disease across all the age groups except for those aged 0-49 years. The subjects in a particular age group suffering from autoimmune thyroid disease were higher than those not suffering from the autoimmune thyroid disease. However, the patients in higher age groups (40-49, 50-59), suffering from autoimmune thyroid disease did not have higher levels of T4 compared to those in a lower age groups (<29, 30-39). There existed no visible linear trend. However, when these differences were tested for significance using the Wilcoxon rank-sum test, they were not statistically significant at 5% level of significance see Figure 5.

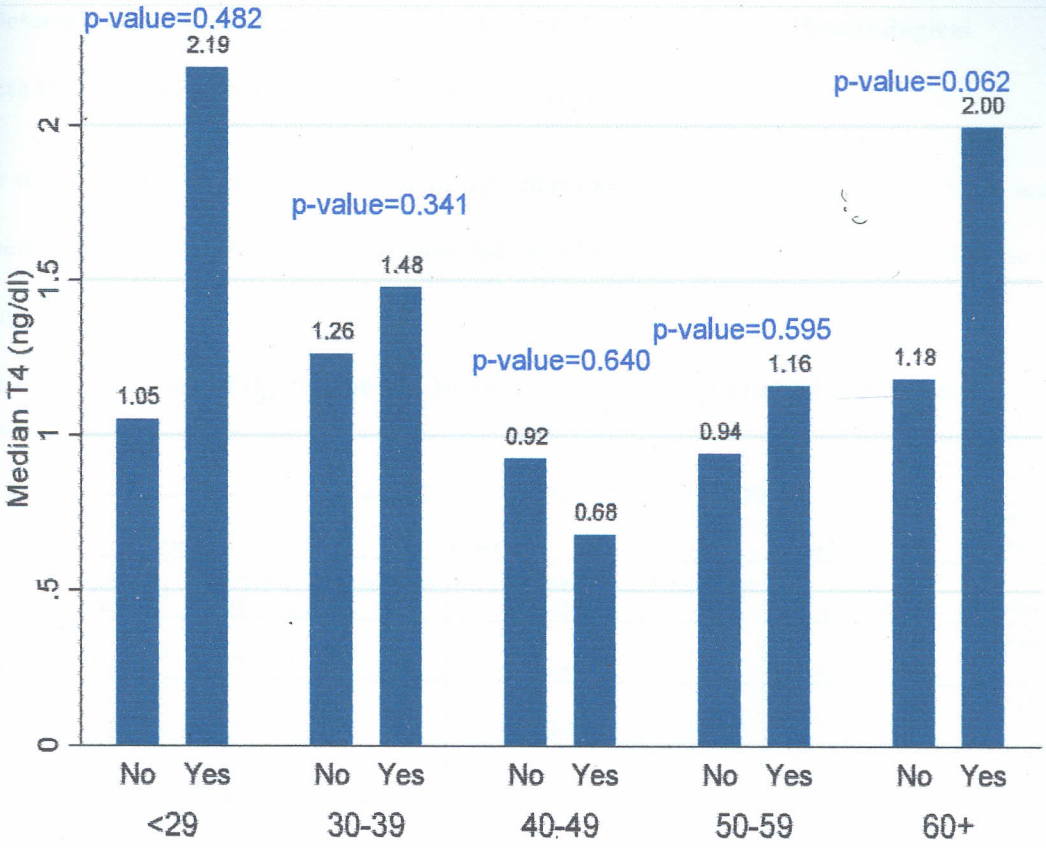

Figure 5: Thyroxine hormone (T4) levels among the immunological patients

4.3 Determination of the Levels of Blood Electrolytes, Proteins and Hematological Indices in Immunological Thyroid Diseases Patients

Table 6 shows the overall distributions of age, hormones, hematological characteristics and biochemical properties which are also stratified by whether immunological thyroid disease is present or absent.

Table 6: Distribution of the hormones, hematological and biochemical properties

			Immunological thyroid disease				
	overall		Present		Absent		Test for difference
Variable	sample size (n)	Median (Q1-Q3)	sample size (n)	Median (Q1-Q3)	sample size (n)	median (Q1-Q3)	p-values
Age	369	40 (33-50)	22	42 (34-48)	347	40 (33-50)	0.990
TSH	333	1.4 (0.7-2.5)	22	1.8 (0.9-2.9)	311	1.38 (0.7-2.5)	0.311
T3	319	1.67 (0.92-2.8)	21	1.8 (1.3-2.7)	298	1.61 (0.9 -2.9)	0.253
T4	327	1.08 (0.7-1.7)	21	1.2 (0.7-1.9)	306	1.07 (0.7 -1.7)	0.489
Urea	268	3.11 (2.3-4.5)	21	3.1 (2.6-4.3)	247	3.12 (2.3-4.5)	0.820
Creatinine	235	54 (48-62)	19	58 (50-67)	216	53 (48-61.8)	0.196
Chloride	252	107.7 (104-111)	21	108.9 (99.5-111)	231	107.5 (104.6-111.0)	0.820
Sodium	252	139 (136.4-144)	21	141.8 (137.7-145.8)	231	139 (136-143.5)	0.183
Potassium	252	4.4 (4.1-4.6)	21	4.5 (4.3-4.5)	231	4.4 (4.1-4.7)	0.732
WBC	252	5.2 (4.1-7.0)	23	4.7 (4.2-7.6)	229	5.2 (4.1-7.0)	0.985
RBC	252	4.6 (4.4-4.8)	23	4.6 (4.4-4.8)	229	4.6 (4.4-4.8)	0.512
HB	252	13.1 (11.6-13.9)	23	12.5 (11.2-13.8)	259	13.1(11.6-13.9)	0.202
Plat	260	292 (224-390)	22	346.5 (221.8-439.3)	238	290 (224.365.2)	0.334

Legend

TSH thyroid stimulating hormone **WBC** White blood cell **Plat** Platelet **HB** Hemoglobin

T3 triiodothyronine hormone **RBC** Red blood cell **T4** thyroxine hormone

Patients with immunological thyroid disease (Yes) had higher profiles of biochemical properties compared to the other group without immunological thyroid disease (No). The difference was in the levels of creatinine levels; the creatinine levels in the two groups were significantly different (**p-value=0.039**). The rest of the biochemical properties were similar for those suffering from autoimmune thyroid disease compared to those not suffering from the condition. The comparisons of the two groups were tested by the Wilcoxon rank-sum test at 5% level of significance see figure 6.

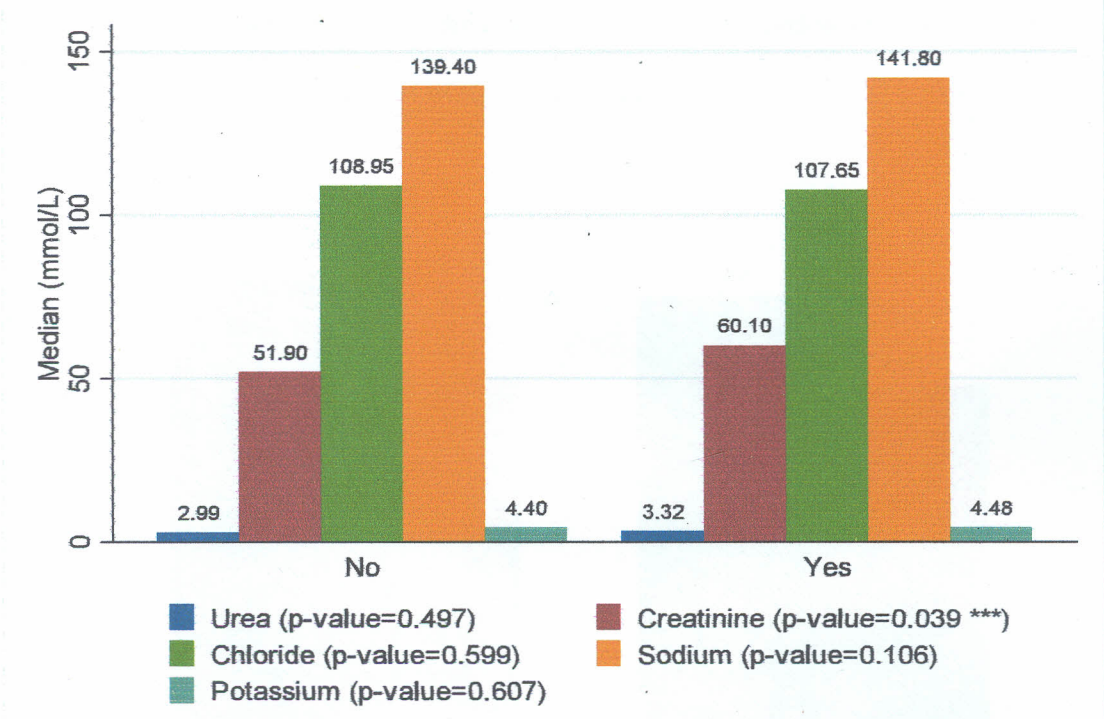

Figure 6: Levels of blood biochemical properties in the immunological patients stratified by whether they had autoantibodies in immunological thyroid disease or not.

In the analysis of the hematological profiles (WBC, RBC, HGB and PLAT) in immunological thyroid disease, the white blood cells and the red blood cells levels in the group suffering from immunological thyroid disease were low. Likewise the hemoglobin levels of the subjects suffering from immunological thyroid were low too. The platelets levels were high for the subjects suffering from the autoimmune thyroid disease.

The white blood cells and the red blood cells levels in the group suffering from autoimmune thyroid disease were low though this did not reach significance level when tested by the Wilcoxon rank-sum test at 5% level of significance, figure 7.

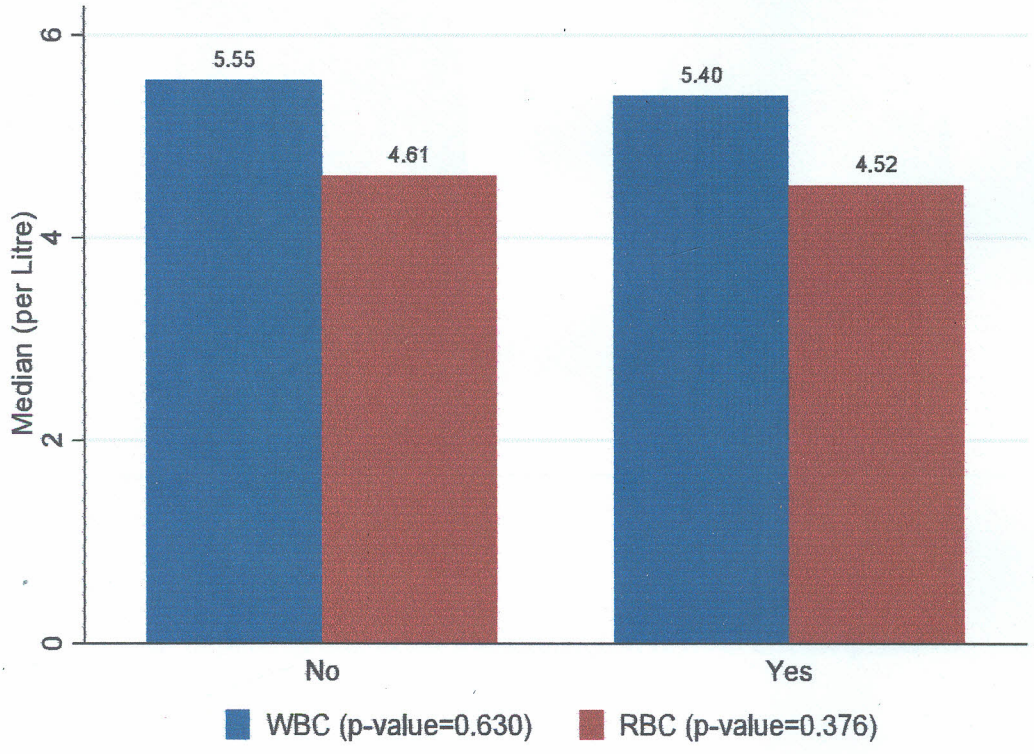

Figure 7: Levels of white blood cells and red blood cells for the immunological thyroid disease patients.

The hemoglobin level of the subjects suffering from autoimmune thyroid disease was also low. However, the difference was not statistically significant for the two groups (p value=0.115), see Figure 8.

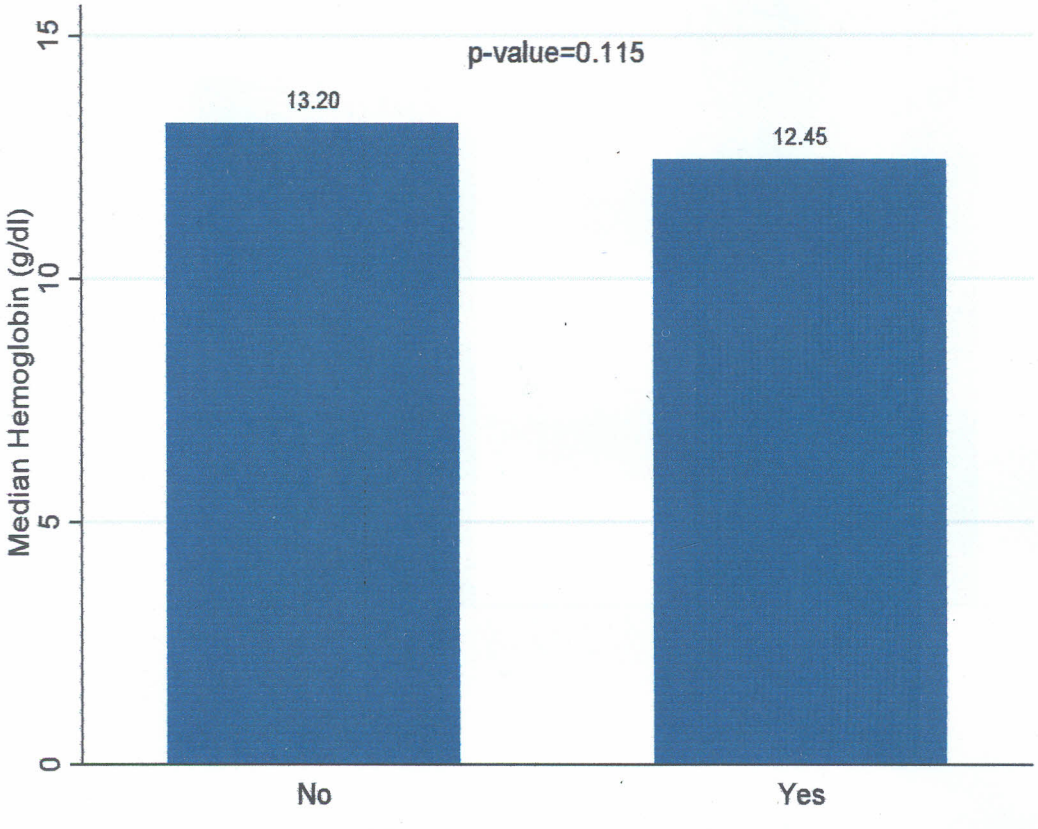

Figure 8: Levels of hemoglobin in the immunological thyroid disease patients.

The platelets levels were high for the subjects suffering from the autoimmune thyroid disease. However, the difference was not statistically significant between the two groups (p-value=0.313), see Figure 9.

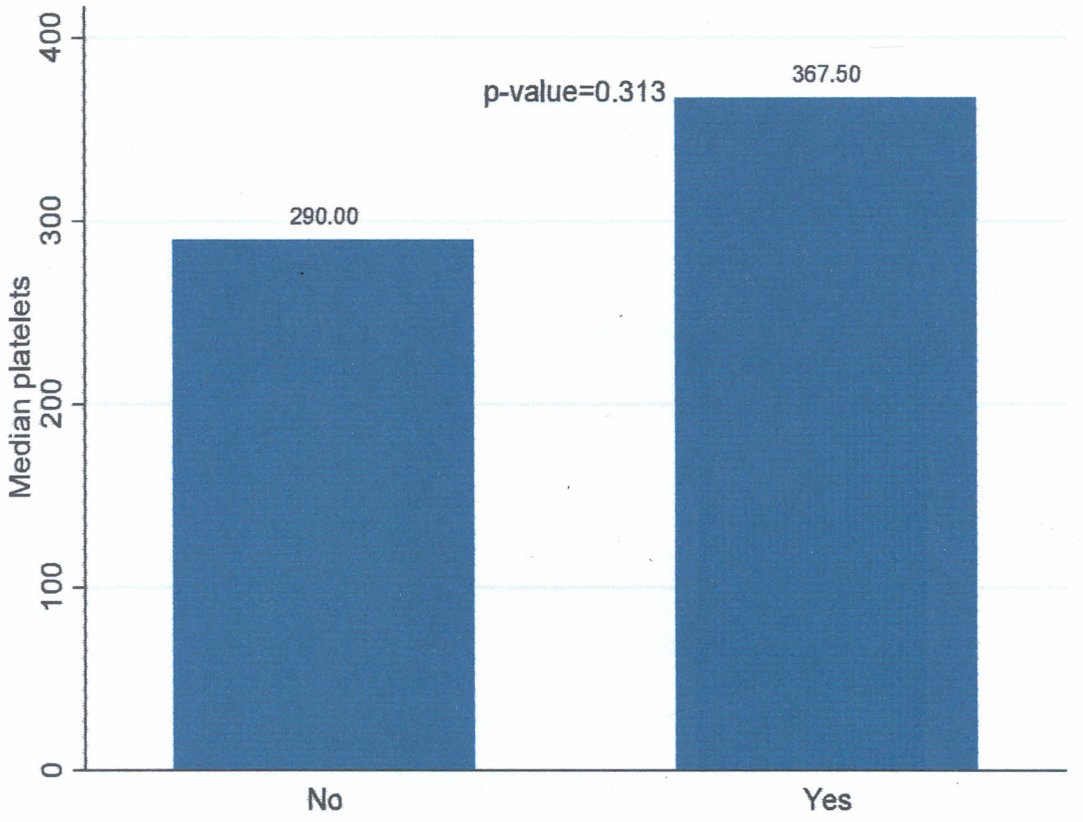

Figure 9: Levels of platelets in the immunological thyroid patients.

CHAPTER FIVE

5.0 DISCUSSION

In general, this study has shown that various thyroid diseases express thyroid autoantibodies. However, the pattern of expression was different when immunological and non-immunological thyroid diseases were compared. Only in immunological thyroid diseases was there significant expression of autoantibodies. Having high levels of T_3 hormone was significantly associated with the development of autoantibodies (p=0.009). Patients with immunological thyroid disease had higher levels of creatinine compared to non-immunological thyroid disease patients (p=0.039). With regard to age (<29, 30-39, 40-49, 50-59, 60+), the significance was only seen in the age group 30-39 only for TSH (0.023) and T_3 (p=0.040), respectively.

5.1 The Prevalence and Histological Patterns of Immunological Thyroid Disease among thyroid disorder Patients.

The study population was mainly made up of females 368 (95%). There was no case of autoimmune thyroid disease that was reported among the male patients though there was no sufficient evidence to justify the association of the autoimmune thyroid diseases with women (p=0.238). However, this may be attributed to the fact that there were very few male subjects compared to females. From the results of this study, the disease is more prevalent in females 368 (95%) than in males 20 (5%). This is in agreement with other studies (Bayliss, 1982; Cotran, 1994).

Colloid goiter represented the largest proportion of the histological diseases as it was seen in 286 (73.7%) of the cases in this study. This prevalence is higher than the earlier reported prevalence (47.7%) in a retrospective histopathological study from 575 histopathological reports over a nine year period in Kenya (Kungu, 1994). This might be due to the difference

in indications for the thyroidectomy and availability of histopathological laboratories. The difference could also be due to a difference in the magnitude of the disease. That is how long one has stayed with the disease and the extent of damage caused by the disease. Colloid goiter is most common throughout the world and most prevalent in mountainous regions (Wolde-gebriel *et. al.,* 1992). This is due to the fact that there is lack of iodine in these regions (Vanderpump *et. al.,* 1992). Lack of iodine leads to decrease in the synthesis of thyroid hormones with a compensatory increase in secretion of thyroid stimulating hormone which in turn may lead to follicular cell hypertrophy and hyperplasia and after sometime enlargement of goiter occurs. Most of the time the enlarged goiter is diffuse in nature but may take the form of discrete nodules. This should be distinguished from enlargement of the gland due to malignancy. The enlargement of thyroid gland can be viewed as homeostatic mechanism to maintain thyroid hormone levels which is due to over stimulation of the tissues (Wartofsky *et. al.,* 1998). It occurs in response to elevated concentrations of thyrotropic hormones. If this compensation is successful, adequate thyroid hormone levels will be maintained and the person will be euthyroid. In some instances, hypothyroidsm may be associated with goiter. Even euthyroid goiters can result in specific problems especially if they are so large as to constrict the neck and interfere with breathing and eating. Other predisposing factors include the presence of anti thyroid substance in the diet, drugs, mineral and bacterial contamination of the drinking water (Lester *et. al.,* 1976). These factors block the synthesis of thyroid hormone which leads to the enlargement of goiter.

The prevalence of adenomas in this study was 51 (13.1%). It represented the second largest proportion of the histological diseases. This prevalence was consistent with the study made by Namba (1976), Gitau (1975) but lower than report made by Kungu (1974). The age and sex distribution of adenoma shows an increase with increase in age. This was consistent with other studies (Bayliss, 1982; Cotran *et al.,* 1994).

Thyroid follicular carcinomas presenting with 18 (4.6%) were seen in this study. This prevalence rate was lower than the 23.3% cases reported by Kungu (1974). Carcinoma of the thyroid are uncommon and mostly in adults although some form may occur in childhood such as papillary thyroid carcinoma (Cotran, 1994). Females are more predisposed than the males and this was noted among patients presenting with thyroid carcinoma in the middle adult age. The sex and age distribution of thyroid carcinoma seen in this study was consistent with other studies (Bayliss, 1982; Cotran *et al.*, 1994; Wahner *et al.*, 1994).

The prevalence rate of immunological thyroid diseases seen in this study is higher (7.2%) than the one reported in earlier studies in Kenya, (Kungu, 1974; Gitau, 1975). The median age of the subjects was 40 (33-50). Stratified by gender, there was no significant difference with regards to age distribution: male 52 (37-73) and female 40 (33-50) with p-value=0.154.

The other histological thyroid disease encountered in this study was thyroiditis 24 (7.2%). This high prevalent rate is higher than the reported prevalence 3% (Kungu, 1974) and 1% (Gitau, 1975). The study sample was made up of 368 (95%) female and out of this, 24 (7.2%) were females. There was no case of thyroiditis in males. This was also seen in other studies (Bayliss, 1982).

Thyroid abscess is a rare condition of the thyroid gland. In this study, the prevalence of thyroid abscess was 0.3%. This presented the least of thyroid pathologies seen in this study. The ability of thyroid gland is to resist infection which is well known and the infection in thyroid gland is rare. Thyroid abscess represents only 0.1 to 0.7% of surgically treated thyroid pathologies (Wartofsky, 1998).

The other thyroid pathology found in this study was thyroid cyst, with the prevalence rate of 8 (2.1%). The prevalence of thyroid cyst has not been reported by other studies in Kenya. The

cystic portion of thyroid is considered to be caused by hemorrhage and subsequent degeneration of preexisting nodules (Mekones, 1996). The cystic changes in metastatic lymph nodes occur in certain types of tumors, site-specific phenomenon that mostly happens in the lymph nodes of head and neck region. Although other thyroid diseases like hyperthyroidism and thyrotoxicosis were not described in this study, it does not mean they are uncommon (Vanderpump *et al.*, 1995).

5.3 Association between Pituitary and Thyroid Gland Hormones (TSH, T3, T4) and Thyroid Autoantibody Levels in Immunological Thyroid Diseases

Of the 388 cases examined, the proportion of patients with thyroid auto antibodies present was 175 (45%). Out of these 175 subjects with auto antibodies present, 11 (6.3%) had autoimmune thyroid disease. This represents 46% among those subjects suffering from autoimmune thyroid disease. The median test showed that there was only statistically significant difference in T3 hormone. This implies that having higher levels of T3 hormone was significantly associated with development of autoantibody. The rest of the hormones did not show any significant association with the development of autoantibodies though their levels were elevated compared to those without autoantibodies.

The serum TSH levels of the subjects suffering from immunological thyroid disease and have autoantibodies when compared to those without autoantibodies present (non-immunological thyroid disease), stratified by age groups the TSH levels were lower for those aged below 40 and suffering from the immunological thyroid disease. However, these serum levels were higher for those aged above 40 and suffering from the same condition. The T_3 levels were high for those subjects suffering from the immunological thyroid disease across all the age groups except for those aged 50-59 years. The T_4 levels were high for those subjects suffering from the immunological thyroid disease across all the age groups except for those aged 50-59

years. The patients with immunological thyroid disease had higher profiles of T_3 and T_4 compared to the other group without immunological thyroid disease.

The results obtained in this study can be attributed to the fact that thyroid stimulating hormone (TSH) is responsible for maintaining proper levels of thyroid hormones in the body. This is a peptide hormone secreted in the anterior pituitary gland by thyrotrope cells. It is the TSH that stimulates thyroid gland to secrete the T3 and T4 hormones (Daniela, 2001). Production of TSH is controlled by the thyrotropin releasing hormone released by hypothalamus and which acts on the anterior pituitary gland. The antagonist of thyrotropin releasing hormone is somatostatin, which is also produced by the hypothalamus. However, whenever there is any kind of imbalance between these antagonistic hormones, it leads to changes in TSH levels (Vaidya, 2002). Elevated TSH levels normally points towards deficiency of thyroid hormones in the body. This is because secretion of TSH depends on the levels of thyroid hormones in the body. The secretion is normally controlled by a negative feedback mechanism (Daniela, 2001). Thus, if the person has low levels of thyroid hormones in the body, then the TSH does not stimulate thyroid gland adequately to secrete thyroid hormones. This condition is known as hypothyroidism. However, this condition can also arise as a result of deficiency of iodine in the diet. The low TSH levels signify a hyperactive thyroid gland, a condition called hyperthyroidism. Once again, if the pituitary gland indicates that levels of T3 and T4 in the blood are high, then due to the negative feedback mechanism, it tends to suppress the release of TSH. Sometimes, the problem is not only due to an imbalance between TSH levels and T3 and T4 levels. There are certain conditions, such as Hashimoto's thyroiditis or Graves' disease, where a person experiences over-activity of thyroid gland due to other problems like an autoimmune condition.

The volume of the thyroid gland increases slightly with age (Hegedus, 1990). With regard to increase and decrease of serum levels in this study, there are no age-related changes in serum

total or free thyroxine concentrations (Braverman, *et. al.,* 1966). Serum thyroxine-binding globulin concentrations decline slightly, those of transthyretin increase and therefore there is no net change in overall thyroxine binding. T4 clearance does decrease modestly with age, but so does thyroxine production, due to a decrease in TSH secretion (Blum *et. al.*, 1989).

Similarly, serum triiodothyronine concentrations in general, do not decrease with age (Olsen *et. al.,* 1978). However, among groups of patients, some have lower serum triiodothyronine concentrations than do normal young subjects. These older subjects probably have some nonthyroidal illness that reduces extrathyroidal conversion of thyroxine to triiodothyronine (Olsen *et. al.,* 1978). This may explain the decrease in hormonal levels found in this study.

In this study, the association between the immunohistochemistry test results and the presence or absence of the thyroid diseases showed that there exists a statistically significant relationship between the two with a p-value < 0.001. This positivity represents a good supportive immunohistochemical evidence of thyroid autoimmunity and its specificity to thyroid autoantibodies which qualifies the disease to be labelled immunological. Hence this study therefore provides a tool for the analysis of immunological thyroid diseases since thyroid autoantibody testing is not routinely done in Africa and few studies have measured thyroid autoantibodies in Africa patients. Hence the occurrence of autoimmunity and the utility of autoantibody testing is thus unclear. Thyroid autantibodies may be found in a variety of thyroid autoimmune disorders such as thyroid cancer, Hashimoto's thyroiditis and Grave's disease. Their presence suggests that there is autoimmune thyroid involvement and the higher the levels, the more likely that is. A baby born from a mother with autoantibodies have a high chance of developing hypothyroidism or hyperthyroidism.

A certain percentage of patients who are healthy may be positive for one or more thyroid autoantibodies. The prevalence of these autoantibodies tends to be higher in women and tends

to increase with age. This is to say that a person may not have thyroid dysfunction but can have thyroid autoantibodies, and therefore her/his health needs to be tracked over time as she or he is likely to develop the disease in the future. Environmental factors may contribute to the low prevalence of thyroid autoimmunity in Africans (Okosieme *et al.*, 2006).

5.4 Blood Biochemistry and Hematological Profiles in Immunological Thyroid Diseases Patients

Similar results were seen for the subjects who had autoimmune thyroid disease (Hashimoto's thyroiditis and Grave's disease) who were stratified by whether autoantibodies are present or absent. This helped in categorising as to whether they have immunological origin or not. The median test showed that there was only statistically significant difference in creatinine levels between the two groups of patients. This significance was in patients with immunological thyroid disease. This can be attributed to the fact that thyroid dysfunction is known to influence serum creatinine levels by decreasing creatinine clearance and eventually increased creatinine release by muscle cells seems to be responsible for the elevated serum creatinine levels (Birewar *et al.*, 2004) as observed in this study. In contrast, decrease in creatinine levels may be seen in patients with hyperthyroidism. However, the serum concentration of Na^+, K^+, and Cl^- are normal. This is also evident in other studies (Birewar *et al.*, 2004). The patients with auto immunological thyroid disease had higher profiles of biochemical properties. The serum levels were high for those aged below 40 and suffering from the autoimmune thyroid disease. Similarly, these serum levels were high for those aged above 40 and suffering from the same condition. Thyroid hormones (TH) are essential for an adequate growth and development of the kidney (Birewar *et al.*, 2004). Conversely the kidney is not only an organ for metabolism and elimination of TH, but also a target organ of some of the iodothyronines' actions (Birewar *et al.*, 2004).

As far as the white blood cells and red blood cells count reduction are concerned, the reduction in hematopoetic cells indicate that the bone marrow is depressed and that thyroid hormones play an important role in the regulation of the human hematopoesis in the bone marrow. This fact is also evident in other studies (Fein *et al.,* 1979).With regards to white blood cells, triodothyronine (T3) hormone has been proven to be a pre-requisite for normal B cell production in the bone marrow through its regulation of pro-B cells proliferation (Foster *et al.,*1999). The hemoglobin level of the subjects suffering from immunological thyroid disease was low too. This clinically indicates a state of anemia. Other studies have also shown that hypothyroidism causes anemia or hyperproliferation of immature erythroid proginators and the anaemia is usually macrocytic hypochromic anaemia (Horton *et al.,* 1976). The increase in the production of platelet can be attributed to the imbalance in the hematopoesis which is a compensatory mechanism (Foster *et al.,* 1999). These observations have confirmed the association between immunological thyroid disease and hematopoesis and that hematological parameters are altered in this condition.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In determining the prevalence and histological patterns of immunological thyroid diseases, this study concludes that thyroid pathologies are present in Kenya. The prevalence of autoimmune thyroid disease was higher (7.2%) than the previously reported by other studies (2%) in Kenya and Africa at large (Kungu, 1974; Gitau, 1975). The prevalence was higher in females (95%) than in males (5%). Among the histological types of thyroid disease, goiter presented with the highest prevalence (73.7%).

In determining the association between pituitary thyroid gland hormones and thyroid autoantibodies, with regards to age, TSH, T3 and T4 hormones, an association was found in T_3 and TSH and that age between 30-39 was significantly high in immunological thyroid disease patients hence thyroid hormones contribute to the occurrence of autoimmune thyroid disease. Likewise T3 was associated with the development of autoantibodies. The presence of the thyroid autoantibodies was significantly associated with immunological thyroid disease.

In determining the levels of blood electrolytes, proteins and haematological profile in immunological thyroid disease patients, this study concludes that blood electrolytes such as Na^+, K^+, and Cl^- are not significantly altered and hence remained within the normal ranges. The blood protein, creatinine levels are significantly elevated in immunological thyroid disease patients. Likewise, thyroid disorders are not associated with hematopoesis and that RBC, WBC, Hb, and platelets are not significantly altered in immunological thyroid disease patients as compared to the non-immunological thyroid disease patients.

6.2 Recommendations

This study showed that colloid goiter presented with the highest prevalence and that it is the most common histological disease. Studies have also proved that colloid goiter is as a result of iodine deficiency and iodine supplement was found to decrease the prevalence of goiter. Hence this study recommends that iodine supplement to be effected to reduce the prevalence of colloid goiter. This will help reduce the prevalence of this disease, and the resources spent on managing individual goiter cases besides alleviating the social impact of goiter.

This study also revealed that thyroid stimulating hormone (TSH) and triiodothyronine hormone (T3) levels for immunological thyroid disease patients were higher those aged 30-39 years hence thyroid hormones contribute to the occurrence of autoimmune thyroid disease. Likewise T_3 was associated with the development of autoantibodies. The presence of thyroid autoantibodies was significantly associated with immunological thyroid disease. Therefore, this study recommends screening of thyroid disease patients for T_3, TSH and autoantibodies for patients between age group 30-39 which is the mid-adult age and identify cases of autoimmune thyroid disease which would otherwise have been misdiagnosed. The clinical utility of thyroid autoantibodies in Kenya patients requires further evaluation in a wider population.

This study also recommends that creatinine levels should be monitored in thyroid disease patients since the levels are significantly elevated in immunological thyroid disease patients. This would be a useful marker to autoimmune thyroid disease since thyroid dysfunction causes changes in glomerular and tubular functions and laboratory diagnosis of thyroid conditions are made based on the thyroid hormone levels only.

6.3 Suggestions for Further Research

A countrywide study need to be done as this will give a holistic view of the dynamics of autoimmune thyroid diseases in Kenya, provide a basis for designing a novel view for diagnosing and therapeutic intervention for thyroid diseases. Clinical evaluation of thyroid diseases and histopathological study of the specimens need to be done to arrive at a definitive diagnosis.

A wider Kenya/Africa population need to be evaluated for the genetic-environmental interactions that may lead to the development of thyroid autoimmunity. How susceptibility genes and the environmental triggers interact to cause autoimmune thyroid disease is not yet known.

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