ANTI-THYROID ANTIBODIES IN PRIMARY THYROID DISORDERS

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A dissertation submitted in part fulfillment for the degree of Master of Medicine (Human Pathology) in the University of Nairobi. 2004

DECLARATION

I certify that this dissertation is my own original work and has not to my knowledge been presented for a degree in any other university.

Signed: ..... Dr. Julius G.Kuria
01/09/2004

This dissertation has been submitted for examination with our approval as University supervisors.

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DEDICATION

This work is written in memory

of the late

Prof Jasper M .Mumo

TABLE OF CONTENTS

	Page No
Title	i
Declaration	ii
Dedication	iii
Table of contents	iv
List of tables and figures	v
Acknowledgement	vi
Abbreviations	vii
Abstract	1
Introduction &literature review	2
Study justification	12
Aim and objectives	13
Materials and methods	14
Ethical considerations	19
Results	20
Discussion	26
Conclusions	32
Recommendations	33
References	34
Appendices	40

LIST OF TABLES AND FIGURES

	Page No
Table 1a: Age and sex distribution of cases	20
Table 1b: Thyroid functional status of study subjects	21
Table 1c: Thyroid hormone concentration among study subjects	22
Figure 1: Age related distribution of α-TgAbs	22
Figure 2: Age related distribution of α-TPOAbs	23
Table 2: Seropositivity to to antithyroid antibodies by diagnosis and sex	24
Table 3: Antithyroid antibody concentrations	25

ACKNOWLEDGEMENT

I wish to hereby extend my sincere gratitude to all those who made it possible for me to start and complete this work.

1 The late Professor J. Mumo, Department of Human Pathology for his supervisory work, encouragement and guidance. It's sad that he did not live to see the fruits of the work which he had put so much into.

2 Dr A. Amayo, Department of Clinical Chemistry for her supervisory work, encouragement and assistance, patience and support throughout.

3 Dr C. Gontier for his invaluable advice.

4 Mrs. Mary Kimani, Mrs Edith Gitonga and Mr. Kilonzo of the Department of Immunology for their much assistance in the laboratory work, together with Mr Obara of the Nairobi Hospital laboratory for his advice.

5 To all my colleagues for their support and motivation.

6 Finally, to my wife Catherine for her encouragement and support and to our children Joy and Alvis for their silent understanding.

ABBREVIATIONS

Ab	Antibody
CA 2 Abs	Second colloid Antibodies
CFT	Complement Fixation Test
DAI	Diagnostic Automation,Incorporated
ELFA	Enzyme Linked Fluorescent Assay
ELISA	Enzyme Linked Immunosorbent assay
FT3	Free Triiodothyronine
FT4	Free Thyroxine
Ig	Immunoglobulin
IU	International units
KNH	Kenyatta National Hospital
RIA	Radioimmunoassay
SPR	Solid Phase Receptacle
SPSS	Statistical Package for Social Scientist
TFTs	Thyroid function tests
TT3	Total Triiodothyronine

TT4 Total Tetraiodothyronine (Thyroxine)

α-TPOAbs Antithyroperoxidase (antimicrosomal) antibodies

Tg Thyroglobulin

α-TgAbs Antithyroglobulin antibodies

TSH Thyroid Stimulating Hormone

TGI Thyroid Growth stimulating Immunoglobulin

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ABSTRACT

Autoantibodies to various components of the thyroid gland have been implicated in the causation of primary hyperthyroidism and hypothyroidism. A study aimed at establishing the association, if any, of the presence of anti-thyroid autoantibodies with primary dysfunctional thyroid disorders was carried out at the Clinical Chemistry and Immunology laboratories of the Kenyatta National Hospital (K.N.H.). The study involved 57 hyperthyroid and 15 hypothyroid patients seen over a period of 6 months, of which 58 were females and 14 were males. The patients were tested for the presence of antimicrosomal and antithyroglobulin antibodies.

Results showed that 36.1% of the patients tested positive for the antithyroglobulin antibodies (α-TgAbs) while 51.4% were positive for the antimicrosomal antibodies (α-TPOAbs). Antimicrosomal antibodies were detected in 57.1% and 50% of the male and female patients respectively (p=0.76). Fifty percent of the hypothyroid patients were found to have the α-TgAbs while only thirty three percent of the hyperthyroid group had the antibody. Twenty three out of twenty six patients (88.5%) who tested positive for α-TgAbs were also positive for α-TPOAbs (p=0.016). Most of those who tested positive for these antibodies were aged 31-50 years (p=0.30 and 0.214 for α-TgAbs and α-TPOAbs respectively).

The findings of this study show that the antimicrosomal and thyroglobulin antibodies are common in patients with primary thyroid disease and should be considered in the pathogenesis of these disorders. Their measurement should be considered in the evaluation of patients suspected of having autoimmune thyroid disease.

INTRODUCTION AND LITERATURE REVIEW

1.1 Physiology of the thyroid gland

The thyroid gland is the largest endocrine gland in man with a weight of about 20 grams in adults. It is located on the anterior aspect of the neck and consists of two lateral lobes connected by an isthmus. It produces three main hormones namely Triiodothyronine (T_3), Thyroxine (T_4) and Calcitonin. The first two are produced by the follicular cells and are concerned with the regulation of metabolism in the body. Calcitonin is produced by the parafollicular cells and is known to have a role in calcium homeostasis in the body. The function of the thyroid gland is primarily regulated by the thyroid stimulating hormone (TSH) released from the anterior pituitary gland.

Disorders of the thyroid gland may be classified into dysfunctional, neoplastic, inflammatory and goitrous. The individual disorder may belong to one or more of the above classes. The dysfunctional disorders, which are of interest to this study, include hyperthyroidism and hypothyroidism. In hyperthyroidism, there is an excess production of thyroid hormones while in hypothyroidism these hormones are deficient. Depending on the aetiology, dysfunctional disorders of the thyroid gland may also be classified into primary and secondary.

1.2 History and background information

Disorders of the thyroid gland have been known to occur for along time. Parry and Graves were the first to report clinical symptoms which were associated with thyroid swelling in 1825 and 1935 respectively (1). The clinical picture of

hypothyroidism was later described by Gull (1). Autoimmune thyroiditis was first described by Hashimoto who reported four patients with goitre in whom the thyroid histological appearance manifested diffuse lymphocytic infiltration, atrophy of parenchymal cells, fibrosis and hurthle cell change (2). The role of autoantibodies in thyroid disease was demonstrated by Rose and Witebsky, who showed the production of thyroid autoantibodies in a rabbit and the subsequent development of chronic inflammatory destruction of the thyroid gland (3). Roitt *et al* (1956) first described the presence of the antibody to thyroglobulin in the serum of some patients with Hashimoto's disease (4).

Disorders of the thyroid gland are a major public health concern in both developing and the developed countries. In areas where there is no iodine deficiency, it is now known that autoimmunity is the commonest cause of thyroid disease (1). In autoimmune disorders of the thyroid, antibodies are formed against various thyroid gland components, products or receptors. The presence of these antibodies in the circulation may not reflect the functional status of the thyroid gland but does help to explain the pathogenesis of various thyroid disorders and inappropriate response to treatment.

Some studies have shown antithyroglobulin (α-TgAbs) and antimicrosomal (α-TPOAbs) antibodies to be present in the sera of about 10% of the normal adult population (1). This is however thought to represent subclinical autoimmune thyroid disease other than false positive reactions (5). Both antibodies show inheritance of a dominant Mendelian pattern in females with a reduced penetrance in males (6). Family studies have repeatedly shown aggregation of α-TgAbs and α-TPOAbs in first degree relatives with autoimmune thyroid disease (7).

The defect that predisposes an individual to autoimmune thyroid disease is still unknown. The proposed mechanisms include a tissue specific defect in suppressor T cell activity, a genetically programmed presentation of a thyroid specific antigen and an idiotype anti-idiotype reaction (5). Each of these defects allows specific organ directed development of auto reactive helper T-cells arising at random throughout life (8). Regardless of the cause. the common outcome is the production of one or more types of autoantibodies. These antibodies are antithyroglobulin antibodies (α-TgAbs), antimicrosomal antibodies (α-TPOAbs), T.S.H receptor antibodies, T.S.H binding inhibiting antibodies. anti T4 or T3 antibodies, Thyroid growth stimulating immunoglobulin (TGI) and the Second Colloid Antibodies (CA-2).

Autoantibodies to a few other organs (e.g. gastric intrinsic factor, islet cells e.t.c.) are found more commonly in patients with autoimmune thyroid diseases by virtue of inheritance of more than one disease susceptibility gene in close proximity to chromosome 6 (5). This is the chromosome which bears the major histocompatibility complex (MHC) and is associated with many autoimmune diseases (3).

1.2.1 Thyroglobulin antibodies (α-TgAbs)

The antithyroglobulin antibodies (α-TgAbs) are formed against thyroglobulin (Tg) a high molecular weight glycoprotein synthesized by the ribosomes of the follicular cells. It is on the thyroglobulin molecule that the amino acid tyrosine is incorporated during the synthesis of thyroid hormones. The thyroid hormones separate from thyroglobulin before their release into the circulation. Thyroglobulin does not normally enter circulation but when it does, α-TgAbs are formed against it (1).

α-TgAbs are usually Abs of the immunoglobulin G (IgG) class but can belong to other Ig classes. Despite the large size of thyroglobulin molecule, Abs to it are

restricted to one minor and two major epitopes (9). α-TgAbs show considerable phylogenetic specificity. Some α-TgAbs have the property of binding thyroxine. Binding of both α-TgAbs and thyroglobulin-antithyroglobulin antibody immunocomplex (Tg-α-TgAbs) to extra ocular muscle membranes has been demonstrated suggesting that this occurrence might trigger the immunological events in the orbit (9).

Studies have shown α-TgAbs to be present in about 60% and 30% of adult patients with Hashimoto's and Graves disease respectively. A study done in Bangladesh showed α-TgAbs to be present in all thyroid patients who tested positive for α-TPOAbs (9).

Antithyroglobulin antibodies are not known to activate the complement system (9). Quantification of α-TgAbs titres carries little diagnostic significance. Positive antibody titres in pregnancy are predictive of postpartum thyroiditis (1). α-TgAbs have also been shown to occur in the course of treatment and follow up of patients with thyroid carcinoma. Increasing concentration of α-TgAbs after total thyroidectomy for differentiated carcinoma indicates metastatic disease (11).

Laboratory methods used for the detection of α-TgAbs include Radioimmunoassay (RIA), ELISA, precipitin test haemagglutination and immunofluorescent assays. (12).

1.2.2 Antimicrosomal Antibodies (α-TPO Abs)

The antimicrosomal antibodies also known as the antithyroperoxidase antibodies (α-TPO Abs) are directed against thyroid peroxidase also called microsomal antigen. This is a membrane bound, glycosylated haemoprotein enzyme

that plays a key role in coupling of iodotyrosyl residues to form thyroxine (T4) and-triiodothyronine (T3) (1). The microsomal antigen is expressed in the microvillar region of the apical thyroid membrane. Large amounts of the antigen are found in thyrotoxic and dyshormonogenetic goitres but very little of it is found in the malignant goitres (9). The antigen is organ specific and Abs show the same phylogenetic specificity reacting only with thyroid tissue from other primates (9).

It has been shown to be present in about 85% of patients with Graves' disease and Hashimoto's thyroiditis (9). It is now known that thyroperoxidase is closely related, if not identical to thyroid microsomal antigen associated with the antithyroid microsomal autoantibodies found in the sera of many patients with autoimmune thyroid disease (13). α-TPOAbs correlate more closely with histologic lesions in hyperthyroidism than α-TgAbs and has a closer relationship with biochemical thyroid dysfunction (9). α-TPOAbs have been shown to be cytotoxic to thyroid cells in tissue culture (5). Methods used for the detection of α-TPOAbs include RIA, ELISA, complement fixation tests (CFT), haemagglutination, and immunofluorescent assays (12). The thyroid disorders associated with the forementioned antibodies include Graves disease, Hashimoto's thyroiditis, postpartum thyroiditis, subclinical hypothyroidism, other thyroiditis e.g. Riedel, thyroid carcinoma and silent thyroiditis.

Low antibody titres occur transiently in some patients after an episode of acute thyroiditis (8). The presence of α-TPO Abs in pregnancy increases the risk of developing postpartum thyroiditis (8).

Autoimmune thyroiditis especially Hashimoto's thyroiditis is more prevalent in persons with various autoimmune and nonautoimmune disorders and some genetic conditions (14). Some of these conditions include autoimmune polyglandular syndrome, Addisons disease, hypoparathyroidism, primary biliary cirrhosis, coeliac disease, Downs syndrome, idiopathic thrombocythaemia, myasthenia gravis, Turners syndrome and vitiligo (5,8). It is important to note that these antibodies may be detectable in patients with the above disorders even in the absence of thyroid disease. Hepatitis C viral infection has also been shown to be associated with thyroid antibodies and subsequent development of Hashimoto's thyroiditis (15).

1.3 Detection of antithyroid antibodies

Various laboratory methods have been used for the assay of thyroid antibodies. They include Enzyme Linked Immunosorbent assay (ELISA). Radioimmunoassay (RIA), haemagglutination, complement fixation tests, immunofluorescence, chemiluminescent assays, cytochemical bioassay and precipitin test. ELISA involves coupling a ligand to an enzyme, which gives a coloured soluble reaction product.

Classically, autoantibodies to thyroid antigens are detected using immunoagglutination and immunofluorescence techniques which are low in sensitivity and specificity. Most of the clinical data has been obtained using the haemagglutination method (16). ELISA and RIA methods are known to provide greater sensitivity and specificity than other methods for autoantibody detecting and for confirming and monitoring of autoimmune thyroid disorders (17). These methods are more suitable for screening and for high volume testing. Haemagglutination is the most widely used method for α-TgAbs assays. The presence of immunocomplexes particularly in patients with high serum Tg levels may however mask the presence of

α-TgAbs (18). Assays for the measurement of such Tg-anti Tg immune complexes have been developed (19) .In this study the ELISA method will be used as it is highly sensitive, specific and relatively more accessible than radioimmunoassay.

1.4 Treatment of autoimmune thyroid disease

Autoimmune thyroiditis is managed by a combination of steroids, neutralizing immunoglobulins and hormone replacement with thyroxine where applicable. Surgery has minimal role in the management of these disorders especially where the antibody titres are high. Hormonal therapy and surgery which are the mainstay in the management of thyroid disease in our setup do not cause a reduction in the titres of the antibodies (1).

1.5 Epidemiology

There is marked variation in the reported frequency and distribution of anti-thyroid antibodies. This variability is due to geographical location and the methods used to detect the presence of anti-thyroid antibodies (19).

In a survey done in patients from a general practice in England, Dingle *et al* found α-TgAbs titres of 1:23 or more in about 16% of women and 4% of men between ages 21 and 30 (20). The prevalence of high levels of α-TgAbs (1:78000 or more) was 4.6% of women and 1.1% of men, which is similar to the frequency of diffuse thyroiditis reported in autopsy findings there (20).

In New Zealand, Couchman and associates (21) found a low prevalence of α-TgAbs titres (ranging from 1:10 to 1:320) in men with no age trend. In women, a prevalence of 2% at age 25 years rising to 15% at age 75 years was found. They also found thyroid cytoplasmic antibodies in 4% of women at age 35 years rising to 20% at age 75 years (21). In the Whickman survey (22) done in N.E. England, α-TgAbs titres of 1:20 or more were found in 2% of the population sampled. The age and sex distribution of all thyroid antibodies studied showed no significant variation with age in men but a marked increase in frequency with age in women.

A study done in Zimbabwe (23) showed that 39% of hyperthyroid patients were positive for microsomal or thyroglobulin antibodies or both. None of the 230 blood donors and goitrous patients had tested positive for either of the two antibodies. This was despite there being significant differences between the two groups with regards to TSH, TT4 and TT3.

Not many studies on autoimmune thyroid disease have been done locally. In

earlier studies done locally on 2820 patients, no cases of spontaneous hypothyroidism or Hashimoto's thyroiditis were reported (Gitau (24), Kungu (25), Mc Gill (26) and Kennedy (27)). In his 1980 study, Muturi found that 5 out of 116 goitrous patients (4.3%) had positive antibody tests (α-TgAbs, α-TPOAbs) while only 1.4% of the control patients had tested positive for the antibodies (28).

In a 1979 study, Bowry and Radia studied 132 cases of thyrotoxicosis and found that 54% and 12% of these patients had α-TPOAbs and α-TgAbs respectively. The incidence of antimicrosomal Abs in our population was found to be similar to that of the Caucasian population (29).

Studies have also indicated that about 10% of mainly postmenopausal women have symptomatic autoimmune thyroiditis and up to 5% also have evidence of a minor degree of thyroid failure reflected by raised TSH levels (1). However antithyroglobulin antibodies are less frequently detected in children with autoimmune thyroid disease as compared to antimicrosomal antibodies (1). It has been noted that higher antibody titres are found in patients with Hashimoto's thyroiditis. A study on the association between α-TPOAbs, TSH and development of thyroid failure showed that only those with markedly elevated levels of α-TPOAbs and TSH developed thyroid failure (30). However quantification of the antibody titre carries little prognostic implications (4).

Thyroid antibodies have been seen to be independent markers for pregnancies at risk for loss (13). Women who have antithyroid Abs miscarry at approximately twice the rate of women who have no antithyroid Abs. Individual levels of α-TgAbs and α-TPOAbs are similarly related to the increased miscarriage rate with no evidence of antibody specificity. Treatment with intravenous immunoglobulin has been shown to result in an improvement in pregnancy rates after in vitro fertilization or embryo

transfer (13). A study carried out on women attending an infertility clinic showed that infertile women have a higher incidence of α-TPOAbs than their control counterparts (1).

STUDY JUSTIFICATION

Diseases of the thyroid gland form a significant portion of our disease burden. With the iodinization of table salt consumed in most countries, the occurrence of iodine related thyroid disorders has markedly declined. A high proportion of thyroid disease in our country may bc due to autoimmunity.

Reports from many parts of the world implicate autoimmunity in the causation of many thyroid disorders. More sensitive and specific methods for detection of anti-thyroid antibodies have been introduced. No local study has been done recently to assess the situation using the newer methods. The results obtained will provide evidence for the current status of autoimmune thyroid disease which may have implications on patient management.

AIM AND OBJECTIVES

The aim of this study was to establish whether there is a relationship between antithyroid antibodies and primary thyroid disease in patients seen at the Kenyatta National Hospital.

Specific objectives

1. To determine the presence of antimicrosomal antibodies in patients with primary thyroid disease.
2. To determine the presence of antithyroglobulin antibodies in patients with primary thyroid disease.
3. To analyze the relationship between autoantibody levels with levels of TSH and thyroxine in patients with primary thyroid disease.
4. To make appropriate recommendations on the management of primary thyroid disease based on the obtained results.

MATERIALS AND METHODS

4.1 Study design

This was a hospital based descriptive cross-sectional study done at the Kenyatta National Hospital (KNH). It was carried out at the Clinical Chemistry and Immunology laboratories of the K.N.H. Kenyatta National Hospital is situated off Ngong Road about 3 km from Nairobi city centre. It is the largest teaching and referral hospital in East and Central Africa. The study was carried out on samples from patients who were sent to the Clinical Chemistry laboratory (K.N.H.) for the determination of their thyroid function.

4.2 Subject definition

Inclusion criteria

- Male and female patients aged 15 to 60 years who had either of the following:

1. TSH levels of less than 0.32 mIU/L and TT_4 level of more than 11ug/dL or FT_4 level of more than 2.3ng/dL (primary hyperthyroidism).
2. TSH levels of more than 5.5 mIU/L and TT_4 levels of less than 4.5ug/dL or FT_4 level of less than 0.8ng/dL (primary hypothyroidism)
3. Informed consent from the patient.

Exclusion criteria;

1. Discordant Thyroid Function Test results.
2. History of thyroid surgery or use of radioiodine.

3. Known or suspected cases of congenital hypothyroidism.

4. Pregnancy

5. Failure to get informed consent from the patient.

4.3 Sample size

Previous studies done locally and regionally have demonstrated the presence of α-TgAbs and α-TPOAbs in between 4.3% and 54% of patients with primary thyroid disorders (23.28.29). 50% will be taken as the proportion of the target population assumed to have the above thyroid antibodies.

This Sample size was calculated using the formular:

$$N=Z^2pq/d^2$$

where:

N=desired sample size

Z=standard normal deviate at the required confidence level

P=proportion of the target population estimated to have the characteristics being measured.

q=1-p

d=level of precision

The confidence interval was set at 90%.

Hence at 90% confidence limit (Z=1.645), the representative sample size is;

$N=(1.64)^2(.50)(.50)/(.1)^2=67.24$

The confidence interval was set at a lower level because of feasibility reasons. If set at 95%, the sample size could have been much bigger with great financial and temporal implications. However 50% was used as the target population with the antibodies so as to get the largest possible sample size at 90% confidence interval.

4.4 Recruitment of the subjects

Patients sent to the laboratory for TFTs were approached by the researcher for recruitment into the study. Those who agreed to participate signed the consent form (appendix 3) and a questionnaire was filled (appendix 1). Subjects with primary thyroid disease were identified only after the TSH and the TT_4 (or FT_4) levels had been determined. It is on these subjects that the antibody assays were done.

4.5 Laboratory tests

Five mls of blood was collected from the antecubital veins into red top evacuating containers (vacutainers). Serum was prepared by allowing blood to clot before being centrifuged at 1000g for 5 minutes. Serum was divided into two equal aliquotes for TFTs and antibodies testing. Serum for the antibody tests was stored at -80 degrees Celsius till enough samples could be collected.for batch analysis. Assays for Thyroid Stimulating Hormone and thyroxine levels were done immediately using the miniVIDAS machine (Bio Merieux) which applies the automated Enzyme Linked Fluorescent Assay (ELFA) technique (appendix 4).

Before being used for the antibody assays, samples were removed from the freezer and kept at room temperature for about 10 hours to come to room temperature. All reagents were removed from the refrigerator and allowed to come to room temperature before use. The antibody tests were run in batches using ELISA method (appendix 2). The absorbance of each test run was read using an automated ELISA

microwell reader (Organon Technika). For each of the absorbances, an index value was calculated. A lot specific exponential regression analysis table provided by the manufacturer was then used to convert the index value to international units (IU) per ml. Index values of less than 0.91 were regarded as negative results. The Diagnostic Automation, Inc.(DAI) ELISA kits were used for the antibody assay. All batches were run with positive controls, negative controls and the standard reagent provided in the kit.

For each of the antibodies, titres were indicated and classified as follows:

Index value	Interpretation
≤0.90	Negative
0.91-1.10	Equivocal
≥1.10	Positive

Only index values of 1.0 and above could be converted using the conversion tables provided by the manufacturer. Index values of 0.91 to 1.09 were not encountered in this study hence there were no equivocal results.

4.6 Data collection and analysis

The data was collected on pre-coded questionnaires (appendix 1). The data was then tallied and summarized on tables and bar graphs, illustrated using ratios and percentages. Data analysis was done using the Statistical package for social scientists (SPSS) computer program and non-parametric chi-square tests. The significance level was set at 0.05.

4.7 Quality Assurance

- ***Reducing pre-analytical errors:***

  -Serum for the antibody assay was separated immediately and stored at –80 degrees Celcius until analysis was done.

  -Repeated freezing and thawing was completely avoided.

  -Lipaemic, haemolysed and icteric samples were not used. In this study, only one sample was found to be haemolysed and was discarded.

  -Reagents were stored as recommended by the manufacturer.

- *Internal quality control:*

  - Immunoassays were run in batches.
  - Batches were run with positive and negative controls which were provided by the manufacturer.
  - If the control values were not within their respective ranges, the test was considered invalid and had to be repeated.

ETHICAL CONSIDERATIONS

The research was undertaken after the KNH ethical and research committee had granted permission for the study to be carried out (appendix 5). Blood samples were collected after the purpose of the study was explained to the subjects who then consented to participate. Blood taken from the patients was used for the purpose of this study only. Residual serum from blood samples collected for thyroid function tests was used for this study. Patients' records were treated with utmost confidentiality. Patients did not suffer delay in the sense that their specimens were processed and the reports of their TFTs were released in good time to assist in the management. Results of the antibodies tests were sent to the doctors to assist in the management of the patients.

RESULTS

Between June and November 2003, seventy two (72) cases of primary thyroid disease were recruited into the study. Fifty seven were hyperthyroid and fifteen cases had hypothyroidism.

Table 1a

Age and sex distribution of cases

Age (years)	Male	Female	Total
< 20	0	3 (4.2%)	3 (4.2%)
21 - 30	2 (2.8%)	4 (5.6%)	6 (8.3%)
31 - 40	5 (6.9%)	19 (26.4%)	24 (33.3%)
41 - 50	5 (6.9%)	22 (30.6%)	27 (37.5%)
51 - 60	2 (14.3%)	10 (13.9%)	12 (16.7%)
Total	14 (19.4%)	58 (80.6%)	72 (100%)

Most of the patients (80.6%) were females. The majority (70.8%) were in the 31 – 50 years age group. Only three patients were below 20 years of age, the youngest being an 18 year old female. The oldest patient was a 58 year old female.

able 1b.

roid functional status of study subjects

gnosis	Males	Females	Total
erthyroid	10 (13.9%)	47 (65.3%)	57 (79.2%)
pothyroid	4 (5.6%)	11 (15.3%)	15 (20.8%)
al	14 (19.4%)	58 (80.5%)	72 (100%)

main thyroid abnormality among the study subjects was hyperthyroidism
ounting for 79.2% of all the cases. There was a preponderance of females who
ounted for 82.4% and 73.3% of the hyperthyroid and hypothyroid subjects
pectively.

Table 1c

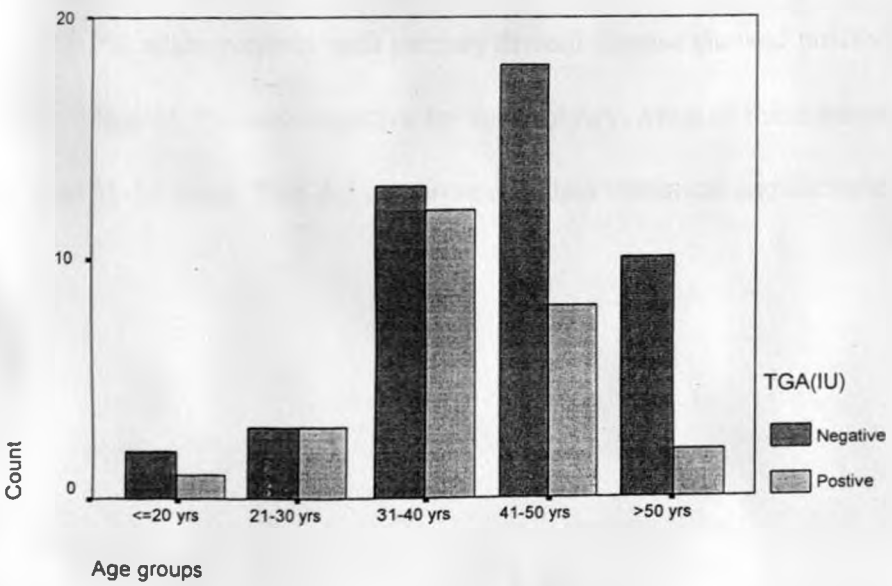
Thyroid hormone concentrations among study subjects

Diagnosis	TSH (mIU/L) Mean(range)	TT4 (ug/dl) Mean(range)	FT4 (ng/dl) Mean(range)
Hyperthyroid	0.03 (<0.001-0.3)	17.27 (11.8-35.0)	4.98 (2.7-20.4)
Hypothyroid	25.12 (9.3-97.84)	3.54 (2.8-4.1)	0.43 (0.15-0.6)

All the subjects had thyroid hormone profiles in keeping with either primary hyper- or hypothyroidism. There were no cases of discordant thyroid hormone results.

Figure 1

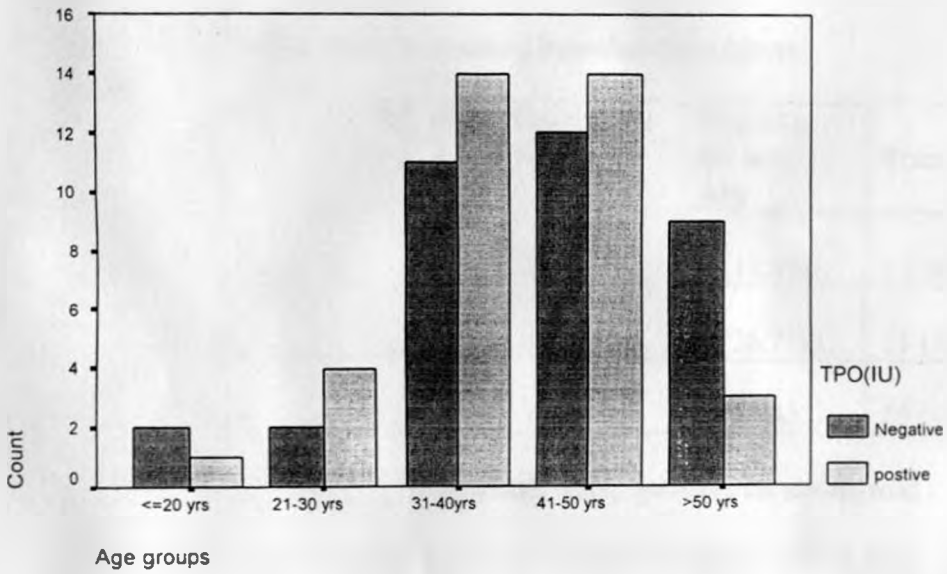
Age related distribution of α-TgAbs seropositivity



Twenty six patients (36.1%) tested positive for α-TgAbs while 63.9% were-negative for the antibodies. Most of these patients (76.9%) were aged 31 to 50 years (p=0.30). Almost half (48%) of the patients aged 31 to 40 years tested positive for this antibody.

Figure 2

Age related distribution of α-TPOAbs seropositivity



51.3% of the patients with primary thyroid disease showed positivity to α-TPOAbs while 48.7% were negative for the antibody. Most of these patients (78.4%) were aged 31-50 years. This did not however attain statistical significance (p=0.214).

Table 2a
Seropositivity to antithyroid antibodies among hyperthyroid subjects

	α-Tg Abs only	α-TPO Abs only	Positive for Both Abs	Negative for both Abs	Total
Males	1 (1.75%)	1 (1.75%)	5 (8.8%)	3 (5.3%)	10 (17.5%)
Females	1 (1.75%)	11 (19.3%)	12 (21.0%)	23 (40.3%)	47 (82.5%)
Total	2 (3.5%)	12 (21.1%)	17 (29.8%)	26 (45.6%)	57 (100%)

Table 2b.
Seropositivity to antithyroid antibodies among hypothyroid subjects

	α-Tg Abs only	α-TPO Abs only	Positive for Both Abs	Negative for both Abs	Total
Males	0	0	2 (13.3%)	2 (13.3%)	4 (26.7%)
Females	1 (6.7%)	2 (13.3%)	4 (26.7%)	4 (26.7%)	11 (73.3%)
Total	1 (6.7%)	2 (13.3%)	6 (40%)	6 (40%)	15 (100%)

Thirty one (54.4%) of the hyperthyroid patients tested positive for antithyroid antibodies, while 60% of the hypothyroid cases showed positivity. These were predominantly against α-TPO Abs. Few cases showed reactivity against α-Tg Abs alone. Among the hyperthyroid subjects, there was a significant difference in positivity to α-Tg Abs between males and females ($p=0.049$). Although more females also tested positive for α-TPO Abs, the difference was not significant ($p=0.43$).
In the hypothyroid group there was no significant difference in reactivity to the antibodies between males and females. Twenty six (45.6%) of hyperthyroid and six (40%) of hypothyroid cases showed negativity to both antibodies. Most of the patients (85%) who showed positivity for α-TPO Abs were also positive for α-Tg Abs ($p=0.016$).

Table 3

Antithyroid antibodies concentrations

Antibody	Diagnosis	Maximum value (IU)	Mean value (IU)
α-TgAbs	Hyperthyroidism	270	96.26
	Hypothyroidism	330	152.14
α-TPOAbs	Hyperthyroidism	688	261.76
	Hypothyroidism	598	240.25

Among the patients who tested positive for the antibodies, levels of α-TPOAbs ranged from 21 to 688 IU while those of α-TgAbs ranged from 20 to 330 IU. TSH values in hyper and hypothyroid subjects were correlated with the antithyroid antibody concentrations. No correlation was found between the antibody titres and thyroid hormonal disturbances ($p=0.189$; $p=0.774$ for α-TgAbs and α-TPOAbs respectively).

DISCUSSION

Primary thyroid disorders are encountered in people of all ages worldwide. Patients with primary thyroid gland disorders present with either features associated with disturbed thyroid hormone production or a clinically detectable goitre. No data is available on the prevalence of primary thyroid disorders in our population. However, in a prevalence survey done in the United States between 1988 and 1994, 5.9% of the American population was found to suffer from thyroid disorders (31).

In the current study, primary hyperthyroidism was more prevalent than primary hypothyroidism with the ratio of hyperthyroid to hypothyroid patients being 3.8:1. This finding is contrary to what is reported in the literature whereby hypothyroidism is three times more common than hyperthyroidism (32). In the American survey, hypothyroidism was present in 4.6% of the American population while only 1.3% of the population had hyperthyroidism (31). This difference may be due to the fact that in this study, only patients with primary thyroid disorders were considered unlike in the other studies where all patients with thyroid hormone disorders were considered. Patients who had hypothyroidism following thyroidectomy or other treatment for hyperthyroidism and those with congenital hypothyroidism were also considered in the other studies. Also, this study was hospital based unlike the American survey which had recruited its cases from the general population. In the same study, patients with subclinical thyroid disease were also included.

The female to male ratio of patients with primary hyperthyroidism in this study was found to be 4.7:1. This is lower than the ratios of 8:1 and 10:1 which have been reported elsewhere (32,33). This difference may have arisen because of the relatively

smaller sample size used in the current study. Eighty percent of patients in the current study were aged between thirty one and fifty years. This compares with what has been found in other populations where hyperthyroidism has been shown to be more prevalent in persons in the third to the sixth decades of life (33,34).

In the current study, primary hypothyroidism also shows a female preponderance with a female to male ratio of 11:4. This is lower than the female to male ratios of 10:1 and to 14:1 which are described in the literature (32,35). The age distribution of patients with hypothyroidism in this study is however similar to that found in the literature whereby most of the hypothyroid patients are reported to be in the 30 to 60 year age groups (33).

Autoimmune thyroid diseases are among the most common autoimmune disorders in man and the presence of antimicrosomal antibodies (α-TPOAbs) is the hallmark of disease activity (32). Another important marker for autoimmune thyroid disease is the antithyroglobulin antibody (α-TgAbs).

The antithyroglobulin antibody (α-TgAbs) was the first antibody to be described in autoimmune thyroid disease. It was described by Roit *et al* in 1956 (4). This antibody is formed against thyroglobulin, a molecule into which the amino acid tyrosine is incorporated during the synthesis of thyroid hormones. In this study, 36.1% of the hyperthyroid patients showed positivity to α-TgAbs. This is higher than what was reported in previous studies done locally. Mc Gill in 1971 found that only 3.3% of patients with thyroiditis tested positive for this antibody (36). In another study done in 1979, 12% of the hyperthyroid patients showed positivity for α-TgAbs (29). The difference in the positivity rate between these studies is likely to be a reflection of the differences in sensitivities of the methods used. Mc Gill (36) used an indirect

immunofluorescence method while Bowry *(29)* used the haemagglutination method, both of which are less sensitive than the ELISA method used in the current study. It may also reflect an increase in the immunological burden due to the high level of environmental pollution and many parasitic and infectious diseases leading to increased production of autoantibodies to various body tissues including the thyroid (37).

A higher proportion of hyperthyroid males (60%) showed positivity for the antibody than hyperthyroid females (27.6%) in the current study (p=0.049). Previous studies which had been done locally did not show the distribution of these antibodies among males and females. In another study, it was reported that this antibody occurs more frequently in females (31) .It is not clear why this difference is there since it is generally known that females suffer more from autoimmune disorders (38). However, the number of hyperthyroid males in this study was small (n=10), and may therefore not be representative of the population. It is possible that a larger study may give different findings. Majority of the hyperthyroid patients who tested positive for this antibody in the current study were aged 31 to 50 years.

More than forty six percent of the hypothyroid patients tested positive for α-TgAbs. A greater percentage of male than female patients tested positive for this antibody (p=0.876). Most of the hypothyroid patients (71%) who tested positive for α-TgAbs were aged 31 to 50 years although the association between α-TgAbs with age was not statistically significant (p=0.30). From the findings of this study, there is no association between the levels of α-TgAbs and the presence of thyroid hormone disturbance (p=0.189). This is similar to what was found in the American survey where the concentration of α-TgAbs was not found to be significantly associated with the severity of hypothyroidism or hyperthyroidism (31).

The antimicrosomal antibodies (α-TPOAbs) are formed against the thyroid microsomal antigen. Fifty seven percent and 50% of the male and female patients studied respectively tested positive for the antibody (p=0.76). This compares with the findings of a recent study done in Asia where 59% and 51.67% of the male and female patients studied respectively tested positive for the antibody (34). Also, most of the patients in the same study were found to be in the 30 to 45 year age group. This is in agreement with what was found in the current study.

In the current study, 51.3% of the hyperthyroid patients tested positive for α-TPOAbs. This compares with the 54% found to have the antibodies in a 1979 study done locally (29). However it was much higher than what was found in an earlier study done locally using indirect immunofluorescence technique (36). Forty nine and sixty percent of the hyperthyroid male and female patients studied respectively were found to have the antibodies, a sex difference that was found to not be statistically significant (p=0.43). In the hypothyroid group, 54% and 50% female and male patients respectively were found to have the antibodies (p=0.52). The findings of the current study show that there is no relationship between the titres of α-TPOAbs and the presence of thyroid hormone disturbances (p=0.774). This study also demonstrates that there is no association between the levels of α-TPOAbs with age (p=0.214).

Most of the patients (88.5%) who tested positive for α-TPOAbs were also positive for the α-TgAbs antibodies (p=0.016). On the other hand, only 62.7% of those who were positive for α-TPOAbs tested positive for α-TgAbs. This compares with what is reported in the literature where up to 100% of patients with α-TgAbs tested positive for α-TPOAbs (1,10). These observations might have arisen from the fact that the two antibodies are polyclonal (39). Again, the number of epitopes in the α-

TgAbs is large as seen with in some of the antisera raised from hyperimmunized animals showing up to 40 epitopes (13). The two antigens have also been shown to share some common epitopes (40). This may explain why most patients with α-TgAbs were also positive for α-TPOAbs despite the kits used having a relatively high sensitivity (100%) and specificity (97.7% and 96.7 % for α-TPOAbs and α-TgAbs respectively).

It is also known that when both antibodies are present, the α-TPOAbs is found more often than α-TgAbs and α-TPOAbs titres are usually higher (1,29). In this study, the mean titres of α-TPOAbs were higher than of α-TgAbs. Also, patients who tested positive for both antibodies tended to have higher titres of α-TPOAbs than of α-TgAbs. In resource poor settings, it may be cost effective to only measure the levels of α-TPOAbs.

It is known that the strength of these antithyroid antibodies' titres does not correlate with the severity of thyroid hormone disturbances (1,31). This is in agreement with the findings of the present study. This implies that a qualitative assay would be as good as a quantitative one in the investigation of autoimmune thyroid disease.

Studies done using modern assay techniques have shown that α-TPOAbs develop in almost all those with autoimmune thyroid disease during some stage in the disease process (32). α-TPOAbs are now considered as a more important marker for autoimmune thyroid disease than α-TgAbs (34,41). In thyrotoxic patients with autoimmune thyroid disease, titres of α-TPOAbs tend to fall once they become euthyroid (42). This also happens among hypothyroid patients who are treated with thyroxine. Doing a follow up study in our population would thus demonstrate how the levels of these antibodies change with treatment. It has been shown that patients with

Grave's disease who have high titres of α-TgAbs and/or α-TPOAbs are more likely to become hypothyroid later (43). A follow up study done in England among on 163 asymptomatic patients with elevated levels of TSH who were also positive for thyroid antibodies (α-TPO, α-TgAbs) showed that these patients developed overt hypothyroidism at the rate of 5% per annum (44). Therefore the determination of these antibodies in patients with subclinical thyroid disease in our population may help us to predict those patients who are likely to develop overt thyroid hormonal disorders. Combination of hormonal profile, microscopic findings and antibody titres would help to make more accurate diagnoses of specific clinical entities like Grave's disease and Hashimoto's thyroiditis.

ELISA and radioimmunoassay (RIA) have been shown to be the most superior methods for assaying these antibodies due to their high sensitivity and specificity (20). Studies done to compare the measurement of α-TPOAbs and α-TgAbs by ELISA with that of the conventional agglutination method have shown that ELISA methods are more specific and cost effective than the agglutination methods. ELISA methods are more rapid as results can be obtained within two to four hours as compared to at least 24 hours required in the conventional haemagglutination method (45). An important limitation of this study is that this study is a cross-sectional study and therefore can not determine how various parameters in the study patients would change with time.

8

CONCLUSIONS

1. Primary thyroid disorders occur more frequently in females than in males in our country.

2. Antithyroglobulin and antimicrosomal antibodies are a common occurrence in patients with primary thyroid disorders.

3. Antimicrosomal antibodies are more common than antithyroglobulin antibodies in both primary hyperthyroid and hypothyroid patients in our population.

4. Most of the patients who test positive for antithyroglobulin antibodies also test positive for the antimicrosomal antibodies and where resources are limited, it may be justifiable to measure the levels of antimicrosomal antibodies only.

5. There is no association between the antibody titres and severity of thyroid hormone disturbances.

RECOMMENDATIONS

1. Determination of α-TPOAbs should be done in female patients with primary thyroid disease.
2. A qualitative assay can be used for the detection of antimicrosomal and antithyroglobulin antibodies in the diagnosis of autoimmune thyroid disease.
3. Follow up studies should be done to monitor how the antibody titres change with successful treatment of primary thyroid disease.
4. Studies correlating the occurrence of the above antibodies with histological diagnoses (eg. Grave's disease) to be done. This would help to make more accurate diagnosis of various autoimmune thyroid disorders.
5. A study to determine the prevalence of these antibodies in children with primary thyroid dysfunction should be done.

REFERENCES

1. Volpe, R. Autoimmune Thyroiditis. In The Thyroid- A Fundamental and a Clinical Text. Lewis, E. B. and Utiger, R. D. 6th ed. 1991:J.B Lippincott Co. p. 921-933.

2. Hashimoto, H. Struma lymphomatosa. *Arch Clin Chem*. 1912; **97**:219-220.

3. Roitts, I. M. Scope of Autoimmune Diseases. In Roitt's Essential Immunology. Roitt,.I.M. 9th ed.1997: Blackwell Scientific.Publications. 399- 410.

4. Roitt, I. M., Ling ,N. R..Campbell, P. N.and Hudson, R.V. Autoantibodies in Hashimoto's Disease. *Lancet*. 1956;**2**:820-821.

5. Volpe, R. Autoimmunity in Endocrine Disease. In Principles and Practice of Endocrinology and Metabolism. Becker, K.L. 2nd ed.1990:J.B.Lippincot Co. p. 1561-1566.

6. Phillips, D. and Mc Lachlans, S. Autosomal Dominant Transmission of Autoantibodies to Thyroglobulin and Thyroid peroxidase. *J of Clinical Endocrinology and Metabolism*. 1990;**70**:742-750.

7. Brix, T.H.,Hansen, P.S. and Kyvik, K.O. Aggregation of thyroid autoantibodies in first-degree relatives of patients with autoimmune thyroid disease is mainly due to genes:a twin study. *J.Clin Endocrinol*. 2004;**329**:34-36.

8. Phuong ,H. N. Autoimmune Thyroid Disease and Pregnancy .*e. Medicine Journal*

2001;**2**: 6.

9. Hall, R. The Thyroid. In Fundamentals of Clinical Endocrinology. Hall, R.and Besser, M .4th ed.1989: Churchhill Livingstone. p. 84-108.

10. Hassanat, M. A.,Runi, M. A., Alam, M. N.and Salam, M. A. Status of Antithyroid Antibodies in Bangladesh .*Postgrad Med J.* 2000;**76**:345-349.

11. Lindstedt, G., Berg, G.,Jansson, S.,Warrin, B. and Nystrom ,E. Clinical Use of Laboratory Thyroid Tests and Investigations. *JIFCC* .1994;**6**:136-141.

12. Whitley, R. J.and Nelson, B. W. Endocrinology part III-Thyroid Function. In Tietz Textbook of Clinical Chemistry. Burtis, A.and Ashwood, R. 2nd ed. 1994: Saunders. p. 1732-1740.

13 Volpe', R. Antibodies to Thyroid Disease. In The Thyroid- A Fundamental and a Clinical Text. Lewis, E. B. and Utiger, R. D. 6th ed. 1991:J.B Lippincott Co. p. 506-523.

14 Fatourechi, V. Demystifying Autoimmune Thyroid Disease. *Postgrad Med.J* 2000;**107**: 1.

15 Vallee, J.,Johanet, C.,Deny, P. and Trinchet, K. High Prevalence of Antibodies to Hepatitis C in Patients with Hashimoto's Thyroiditis. *BMJ.* 1994;**309**:846-7.

16 Cayser, I.,Donarch, D. and Chalmers, S.R. An evaluation of two new haemagglutination tests for rapid diagnosis of autoimmune thyroid disease. *J of Clin Path.*1978;**31**:1147-1152.

17 Roman, S.,Karn, F. and Davies, T.F. Enzyme Linked Immunosorbent microassay and haemagglutination compared for detection of thyroglobulin and thyroid microsomal autoantibodies. *J Clin Chem.*1984;**30**: 246-248.

18 Woeber ,K. A. Subclinical Thyroid Dysfunction. *Arch.Internal Med.*1997;**157**:1065-1068.

19 Braveman, L.E., Roti ,E., Gardini ,E.and Minelli, R. Prevalence of anti-thyroid peroxidase antibodies in serum in the elderly:comparison with other tests for anti-thyroid antibodies.*J Clin Chem.* 1992;**38**:88-92.

20 Dingle ,P. R.,Ferguson, A.,Tubmen, J. and Hall, R. The incidence of TGA and thyroid enlargement in a general practice in N.E. England. *Clin Exp Immun.*1966;**1**:277-8.

21 Couchman ,K G. and Wigley ,R. D. Autoantibodies in the Carleton Population Survey. *J Chronic Diseases.*1970; **23**:45.

22 Whickman, M.,Tumbridge, G.and Caldwell, G. The epidemiology of thyroid Diseases. *J.Chronic Diseases.*1978;**5**:87-89.

23 Chinyanga ,E.A., Benni, A. and Siziya, S. Thyroid Status and the Levels of Thyroid Autoantibodies in the sera of Hyperthyroid and Goitrous subjects. *Central Afr Med. Journal.* 2000;**45**:389-90.

24 Gitau, W. An Analysis of Thyroid Diseases seen at K.N.H. *E.Afr Med J*

.1976;**52**:564-569.

25. Kung'u, A. The pattern of thyroid disease in Kenya. *E.Afr.Med.J.*1974 ,**51**: 499.

26. Mc Gill, P. E.and Svenson ,E.O. Plasma inorganic iodine values in subjects from Kiambu and Nyeri districts in Kenya. *E.Afr.Med.J* .1972;**49**:27.

27. Kennedy, J. Surgical goitres in Glasgow and Nairobi. *E.Afr.Med.J.* 1970;**47** :73.

28. Muturi, I. L.M. A study of nontoxic goitre disease at K.N.H ,*MMed dissertation.*1980;*UON.*

29. Bowry ,T. R. and Radia, R. Thyroid autoantibody profile in African Thyrotoxicosis. *E.Afr.Med.J* .1979;**56**:640-645.

30. Rosenthal, J., William ,C. H.,Mark, J.and Garry, P.J. Thyroid failure in the elderly. *JAMA* .1987;**258**:209-213.

31. Hollowell, G.J.,Norman, W.S.,Hannon, W.H.,Spencer, C.A.,Braverman, L. *et al.* Serum TSH, T4 and Thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J of Clin Endocrinology & Metabolism.* 2002;**87**: 489-499.

32. .Mc Gregor, A.M. The Thyroid Gland and Disorders of Thyroid Function. In Oxford Textbook of Medicine. Weatherhall, D. ,Lendingham, J. and Warell, D.A. 3rd ed.1996 :Oxford.p.1603-1621.

33. Walker, B.R. and Toft, D.A. Endocrine disease. In Davidsons Principles and

Practice of Medicine. Haslett, C.,Christopher, H.,Chilvers, E. R.,Boon, N.A.,Nicki, R *et al.* 19th ed .2002: Churchhill Livingstone.p. 689-704.

34. Mahmoud, S.,Islam, M.M.,Siddiqui, N.I.,Hossam, G.A. and Akter, N. Prevalence of antithyroid antimicrosomal antibodies in thyroid patients of endemic goitre area. *Mymensingh medical journal.* 2004;**13**:4-10.

35. Larsen, P. The Thyroid. In Cecil Textbook of Medicine. Wyngaarden, J.B.,Lloyd, H. and Bennett, J.C. 19th ed.1992:Saunders.p.1261-1279.

36. Mc Gill, P.F. Thyrotoxicosis in the Africans; Clinical and Immunological Observations..*Brit.J.of.Med.* 1971;**2**:679-70.

37. Weetman, A. P. and Mc Gregor, A. M. Autoimmune Thyroid Disease: Developments in our understanding. *Endocr.Rev.* 1984; **5**:309-50.

38. Kast, R. E. Predominance of Autoimmune and Rheumatic Disease in Females. *J.Rheumatol.* 1977;**4**:288-92.

39. Nye, L..Roit ,I. M.and Decarvalho ,L.P. An investigation of the clonality of human autoimmune Thyroglobulin antibodies and their light chains. *Clin Exp Immunology.* 1981;**46**:12.

40. Naito, N. and Saito, K. Antithyroglobulin antibodies in sera from patients with chronic thyroiditis and from healthy subjects-differences in cross-reactivity with thyroid peroxidase.*Clin Exp Immunology.*1990;**80**:4-5.

41. Tietz, N. W..Clinical Guide to Laboratory Tests.3rd ed.1995: Saunders (Ed).p.589.

42. Jannson ,R.,Karlson, A. and Dahlberg ,P.A. Thyroxine, Methimazole and thyroid antimicrosomal antibodies in Hashimoto's Thyroiditis. *Brit.J.of.Med* .1985;**290**:11-13.

43. Irvine ,W.J. and Stuart, A. G. Prognostic significance of thyroid antibodies in the management of thyrotoxicosis. E&S Livingstone Ltd (Ed). 1961;London.p 118.

44. Turnbridge, W. G.,Brown, M. and Freud, J. M. Natural History of Autoimmune thyroiditis. *Brit J of Med.* 1981;**282**:258-262.

45. Stephen, D.C.,Thomas, N.,Selvakumar, R.and Seshadu, M.S. Thyroid autoantibody measurement by EIA. *Indian Journal of Clinical Biochemistry.* 1997;**12**:35-39.

APPENDICES

Appendix 1

QUESTIONNAIRE

ANTI THYROID ANTIBODIES IN PRIMARY THYROID DISEASE

1. Study No of patient □□□

2. O.P/I.P No of patient □□□□□□□□

3. Age of the patient □□ years

4. Sex 1. Female □ 2. Male □

5. Clinical diagnosis TICK BOX

1.Hyperthyroidism □

2.hypothyroidism □

3.Goitre □

4.Others □

6. Date of blood collection □□□

7. Date of analysis □□□

8. Level of TT4

----------ug/dl

9. Level of FT4

----------ng/dl

10. Level of TSH

--- ----------mIU/L

11. Presence of α-TPO Ab TICK BOX

1.Negative ☐

2. Positive ☐

12. Presence of α-TgAb TICK BOX

1.Negative ☐

2. Positive ☐

Appendix 2

ELISA method

DAI- antimicrosomal and antithyroglobulin ELISA kits

The ELISA kits used in this study for the determination of antimicrosomal and antithyroglobulin antibodies apply the same principle and have similar procedural steps.

The DAI test kit is an ELISA kit to detect Immunoglobulins G, M and A to antithyroglobulin (or antimicrosomal) antigens. Purified α-TgAbs (or α-TPO Abs) are attached to a solid phase micro assay well. Diluted test sera (1:21) are added to each well. (The diluent contains a buffer,tween 80 and 0.1% proclin as a preservative). If Abs are present that recognize the antigen, the antigen-antibody complexes are formed. Wells are incubated at room temperature for 30 minutes and then washed to remove the unbound Abs. A conjugate is then added to each well. The conjugate (containing horseradish peroxidase conjugated anti-human IgG,IgM and IgA in a buffer) binds to the above complexes if present. After incubation at room temperature for 30 minutes, wells are washed to remove the unbound conjugate.

A substrate solution (3,3',5,5'-tetramethylbenzidine-TMB) is then added to each well .This is then incubated at room temperature for 15 minutes. If the enzyme is present, the substrate will undergo a colour change (from blue to yellow). The reaction is then stopped by the addition of a stopping solution (concentrated sulphuric acid). The optical density of each well's contents is determined by use of a spectrophotometer (at 450nm) producing an indirect measurement of the specific Ab in the patient specimen. An index value is then calculated from which the actual antibody titre is derived using the provided conversion tables.

Appendix 3

Consent form

ANTI-THYROID ANTIBODIES IN PRIMARY THYROID DISEASE

I_____ of _____

do hereby consent to be recruited into the proposed study(above).The following issues have been clearly explained to me;

- My participation in this study is voluntary and I may withdraw from the study at any time.
- The blood sample obtained from me will be used for the sole purpose of this study and the results obtained shall be communicated to my clinician for the purpose of my treatment.
- That I shall not receive any biased treatment (favours or denials) in regards to my status as a study subject.
- That the results obtained shall be treated with confidentiality.
- My participation in the study will not expose me to any risks since no additional venepuncture will be required and the amount of blood that will be needed for the study is minimal.

Signed---------- Date----------

***Witnessed by*---------- *Date*----------**

Appendix 4

Enzyme Linked Fluorescent Assay (ELFA) technique for the assay of thyroid hormones using the miniVIDAS system

Principle: The miniVIDAS automated system combines the principle of enzyme immunoassay with a final fluorescent detection technique. All reaction steps are performed by the VIDAS instrument. The disposable solid phase receptacle (SPR) serves both as a solid phase and a pippeting device during the assay. Reagents for the assay are all contained in the sealed reagent strips.

Procedure

1. The kit is removed from the refrigerator and allowed to come to room temperature for at least 30 minutes. The specific hormone strip and SPR are removed for each sample, control or calibrator to be tested. The storage pouch is resealed after the required SPRs have been removed.

2. The specific hormone strip and SPR are then placed on the VIDAS preparation/loading tray. The appropriate patient data is then entered to create a work list. The name of the particular hormone to be assayed is then typed to enter the bar code.

3. The calibrator should be run in duplicate or triplicate depending with the hormone to be assayed.

4. The samples, calibrators and the control are then mixed using a vortex before being pippeted into the sample wells (volumes of 100uL or 200 uL are used depending with the particular hormone to be assayed). The SPRs and strips are then inserted into the positions indicated on the screen.

5. Analysis is then initiated as directed by the user manual provided by the manufacturer. All assay steps are automatically controlled by the instrument. Results are obtained in 30 or 40 minutes depending with the hormone being assayed.

6. When the assay is completed, the results are automatically calculated by the miniVIDAS using a calibrator curve which is stored in memory and then printed.

Appendix 5

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.

Tel: 726300-9
Fax: 725272
Telegrams: "MEDSUP", Nairobi.
Email: **KNHplan@Ken.Healthnet.org**

Ref: KNH-ERC/01/1798 **Date: 23 May 2003**

Dr. Kuria G. J.
Dept. of Pathology
Faculty of Medicine
University of Nairobi

Dear Dr. Kuria,

RESEARCH PROPOSAL "ANTI-THYROID ANTIBODIES IN PRIMARY THYROID DISORDERS" (P136/11/2002)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** the revised version of your above cited research proposal.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,

PROF. A. N. GUANTAI
SECRETARY, KNH-ERC

Cc Prof. K.M. Bhatt, Chairperson, KNH-ERC
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