

**CORRELATION BETWEEN CYTOLOGIC DIAGNOSIS AND MEASUREMENTS OF
SERUM THYROTROPIN AND THYROID HORMONES IN PATIENTS WITH THYROID
NODULES**

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DECLARATION

I certify that this is my original work and has not, to the best of my knowledge, been presented for a degree in any other university.

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

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DEDICATION

To my dear mother Jane Chepngeno Kirui, for moral guidance and support throughout my educational life.

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LIST OF ABBREVIATIONS

AFTN.....	Autonomous functioning thyroid nodule
CL.....	Confidence limit
Df.....	Degrees of freedom
ELISA....	Enzyme linked immunosorbent assay
FNA.....	Fine needle aspiration
KNH.....	Kenyatta National Hospital
MEN.....	Multiple endocrine neoplasia
N/C.....	Nuclear cytoplasmic ratio
SD.....	Standard deviation
SOPC.....	Surgical Outpatient Clinic
TBG.....	Thyroxine binding globulin.
TRH.....	Thyrotrophin releasing hormone
TSH.....	Thyroid stimulating hormone (Thyrotrophin)
T3.....	Tri-iodothyronine
T4.....	Thyroxine
FT3.....	Free tri-iodothyronine
FT4.....	Free thyroxine
RAIU.....	Radio-active iodine uptake

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SUMMARY

Introduction. The thyroid is an endocrine gland involved in the homeostatic regulation of man by means of its products, the hormones tri-iodothyronine (T3), and thyroxine (T4), which is in turn regulated by thyroid stimulating hormone (TSH), produced by the anterior pituitary gland. The basic morphologic unit is the follicle, composed of follicular cells which produce the hormones T3 and T4. The diseases affecting the thyroid are classified into benign and malignant lesions or disorders of thyroid function, hyperthyroidism and hypothyroidism. Thyroid dysfunction is evaluated by measuring serum TSH, T3 and T4 which will establish euthyroidism, hyperthyroidism and hypothyroidism. Fine needle aspiration (FNA) is the diagnostic test of choice in determining whether a nodule is benign or malignant. It has not been established, whether the various lesions diagnosed by FNA have differing hormonal profiles, and this is the main research problem being investigated.

Aims and Objectives. The aim of the study was to establish cytologic diagnosis of thyroid nodules and to determine the serum total T3, T4 and TSH levels in the same patients. It also sought to correlate the hormonal levels to the cytologic findings and to make recommendations on the use of FNA and hormonal analysis for improved diagnosis and patient management.

Study Design. A cross-sectional study of 42 patients with thyroid nodules was done at Kenyatta National Hospital (KNH) between June and August 2001.

Study area and population. The study involved patients who presented to the Surgical Outpatient Clinic (Thyroid and FNA clinic) with a thyroid nodule at KNH over the same period. Inclusion criteria included any patient who had a nodular or diffuse thyroid goiter, a request for FNA by the attending clinician, informed consent and exclusion criteria included any patient who was on treatment for a thyroid disorder, or refused informed consent.

Methodology. FNA cytologic diagnosis was performed by the investigator and confirmed by a cytopathologist in the Department of Human Pathology. Serum total T3, T4 and TSH levels were performed in the Department of Clinical Chemistry, using the Serozyme ELISA technique.

Results. The data was analyzed to evaluate the correlation between FNA diagnosis and hormonal levels. There were 37 (88.09%) females and 5 (11.90%) males giving a female to male ratio of 7 : 1. The hormonal profile after biochemical analysis showed that 26 (61.6%) patients had a hormonal profile of euthyroidism, 5 (11.5%) had hyperthyroidism, 8 (19%) had compensated hyperthyroidism, 2 (4.76%) had subclinical hyperthyroidism and 1 (2.3%) had subclinical hypothyroidism. Patients with FNA cytological diagnosis of nodular goitre comprised 35 (83.3%), non-diagnostic samples 3 (7.14%), papillary carcinoma, atypia, thyroglossal cyst and thyroiditis 1 (2.38%) each. With a confidence interval of 95% and $p < 0.05$ there was no significant statistical difference of the mean levels of T4 ($p = 0.406$), T3 ($p = 0.311$), and TSH ($p = 0.90$), between and within the various groups of FNA cytological diagnoses.

Conclusion. The study showed there was no correlation between hormonal profile and FNA cytological diagnoses. Both biochemical analysis and FNA biopsy, offered to the clinicians the possibility of early diagnosis of both thyroid function and benign and malignant lesions, and to determine the course of therapy in the management of patients with thyroid nodules.

Recommendation. FNA cytology and thyroid function tests should be reinforced as routine diagnostic procedures at KNH for the diagnosis and management of patients with enlarged thyroid glands and adequate rational resources allocated to the concerned departments and laboratories. There is need to conduct a comprehensive prospective study at KNH to correlate hormonal profile, preferably FT4, FT3 and TSH, with cytology and histology where time, resources and an adequate sample size is considered.

INTRODUCTION AND LITERATURE REVIEW

The normal thyroid gland is composed of two lobes with a connecting isthmus. Microscopically the lobes are divided into numerous lobules, each containing 20 to 40 follicles, which are closed spheres lined by follicular cells and filled with colloid (1). The function of the adult thyroid gland is primarily regulated by thyroid stimulating hormone (TSH) released by the anterior pituitary gland which in itself is regulated by the hypothalamus via thyrotropin releasing hormone (TRH). Thyroid stimulating hormone TSH acts on the thyroid causing follicular epithelial cells of the gland to convert thyroglobulin into the thyroid hormones, thyroxine (T₄) and lesser amounts of tri-iodothyronine (T₃). These thyroid hormones T₄ and T₃ are released into the systemic circulation where most of it is bound reversibly to thyroxine binding globulin (TBG), for transport to peripheral tissues. Thyroid hormones influence every cell in the body, including those in the brain, heart, liver, kidney, skin and bone. They are essential for normal growth, mental development and sexual maturation. Free T₄ and free T₃ levels are in minute concentrations. FT₃ is three to five times more potent than FT₄. These FT₄ and FT₃ hormones enter cells and interact with nuclear receptors that change gene expression and ultimately increase carbohydrate and lipid catabolism and stimulate protein synthesis. The net effect is increase in basic metabolic rate (BMR)(2,3).

Diseases of the thyroid gland usually result in thyroid enlargement (goiter) which can be due to infections, cystic changes, autoimmune diseases, iodine deficiency leading to hyperplasia and neoplasia, with or without thyroid hyperfunction (hyperthyroidism) or thyroid hormone deficiency (hypothyroidism). The World Health Organization (WHO) grading system is used to define a goiter; Grade 0- not palpable or visible goitre even with the neck in the extended position, Grade 1- subjects with a palpable goitre divided into; 1A-goitre detected only by palpation, 1B-goitre palpable and visible with the neck fully extended, Grade 2-goitre visible with the neck in the normal position; Grade 3-very large goitre visible from a considerable distance (4). Allowing for different criteria in various community studies a goiter is generally defined as an enlarged palpable and visible thyroid enlargement which may be diffuse initially but most if not all goitres become nodular with time. Thyroid nodules are frequently detected by ultrasonography or autopsy examination if they are not clinically palpable or visible.

The most common cause of thyroid disorders worldwide is iodine deficiency leading to goiter formation and euthyroidism. In areas not deficient in iodine, autonomous processes are believed to be the basis of most cases of thyroid disease ranging from hyperthyroidism to hypothyroidism. There have been many surveys of the prevalence of goitre in many countries. It is not uncommon with a prevalence of 4 - 10% of the general population upon neck examination and up to 50% of autopsy cases. Although the presence of a nodule raises suspicion for cancer, only 5% are in fact malignant (5,6).

First and foremost a comprehensive history and physical examination is essential. Skill in palpation of the thyroid gland should not be dismissed. Clinicians with little experience in thyroid examination should consider referring patients to thyroid consultants before scheduling any potentially unnecessary and expensive testing. History of external neck irradiation during childhood or adolescence increases the incidence of malignant thyroid nodular disease. This will help direct the investigation and may eliminate the need for some diagnostic tests(7).

Next a sensitive T4, T3 and TSH assay should be done to determine the presence of hyperthyroidism or hypothyroidism. The diagnosis of hyperthyroidism and hypothyroidism can be made by using different methods but the most common ones are serum TSH levels in conjunction with total T4 and T3 levels. Rarely do patients with malignant thyroid nodules have hyperthyroidism or hypothyroidism. Typical reference ranges for serum thyroid hormones and TSH in humans are T4-(67-163 nmol/l), T3-(1.12-2.66 nmol/l) and TSH -(0.6-4.5 uIU/ml). Age -related variations, illness, pregnancy, nutritional changes and various medications may cause normal references ranges to change. The arguments for measurements of TSH, T3 and T4 is strongest before treatment is started(7,8).

A major advance in the diagnosis of the thyroid nodule has been achieved with the perfection and common use of fine needle aspiration (FNA) biopsy. Fine needle aspiration biopsy can be defined as the removal of a sample of cells, using a fine needle, from a suspicious mass for diagnostic purposes(9). It is cost effective and reliable and is now believed to be the most effective pre-operative method available for distinguishing between benign and malignant thyroid nodules. Fine needle aspiration has become an important diagnostic technique that is now appreciated and practiced in the majority of large hospitals throughout the world. Ever since needles and syringes were introduced into the medical field, they were used to aspirate collections of fluids that could be

examined under a microscope. Some of the earliest descriptions of diagnostic applicability of these instruments go back to the 1830s'. The first applications of the aspirated sample pertained to infectious diseases. Greig and Gray (1904) identified trypanosomes in material aspirated from enlarged lymph nodes in-patients with sleeping sickness in Africa using a hypodermic needle and syringe. Sampling of tumours using FNA was first described in the United States of America by Martin and Ellis in 1930. It gained considerable prominence first at Karolinska Hospital in Stockholm by work done by Franzen, Zajicek, and Lowhagen in the 1950's and 1960's. It later gained prominence in the United States of America (9).

Fine needle aspiration (FNA) is now widely used in clinical practice for the investigation of palpable masses such as enlarged cervical and inguinal lymph nodes, breast lumps, thyroid and parotid tumours. It is also used to evaluate cystic and infectious lesions. Non-palpable lesions are usually aspirated under the guidance of imaging techniques such as fluoroscopy, computed tomography, ultrasound and occasionally, such procedures as stereotaxic mammography. For the procedure to be done properly a familiarity with general anatomy is required by a clinician or cytopathologist. The cytopathologist must have a good knowledge of normal cell elements in smears. FNA may encompass practically all tumor types, both benign and malignant and cysts encountered in the general practice of pathology.

Fine needle aspiration (FNA) has several advantages. It is a safe quick investigation with a rapid report and is highly sensitive and specific for the diagnosis of malignancy. It requires little equipment and causes minimal inconvenience to patient and can be performed as an outpatient or bedside procedure. It promotes optimal scheduling of admissions to hospital and cuts down on bed occupancy for diagnostic investigations. It allows pre-operative diagnosis, informed patient consent and planned surgery. It obviates the necessity of frozen section, reduces the incidence of exploratory laparotomy and thoracotomy and allows definitive diagnosis on inoperable patients. Finally FNA has no problems with wound healing, is readily repeatable and highly cost effective. There are few disadvantages of FNA. Practice and skill in aspiration techniques is necessary and a percentage of aspirates are unsatisfactory for cytologic evaluation. Experience is required for accurate interpretation.

There are important principles that should be followed before FNA is performed. The patients clinical history and physical examination if not already known must be secured in advance of the procedure. The section to be aspirated must be palpable or visualized and its suitability for

ration should be assessed. The procedure should be explained to the patient and an informed consent obtained. For successful fine needle aspiration three criteria must be fulfilled.

Aspiration of adequate and representative material

Correct processing of specimen

Informed interpretation and issue of accurate report

The first two of these stages are absolutely vital. No amount of experience on the part of the pathologist can compensate for an unsatisfactory smear. Conventional excisional biopsy has never achieved significant popularity in the diagnosis of thyroid disorders. FNA is essentially non-traumatic. It gives minimum risk and suffering for the patient. Disposable gloves, needles and syringes using aseptic techniques are used. Fine (23gauge) and short (1-1.5 inch) needles are preferably used. Traumatic complications of FNA of palpable lesions are infrequent and always of a minor degree. A hematoma occasionally forms at the site of the aspiration but causes little discomfort. Infections occur in very few cases. Dissemination of tumor cells or cell clusters through needle track is a rare occurrence as several studies indicate (9).

Several FNA biopsy series and reviews have been performed to establish the efficacy of this procedure. Mazzaferri et al(10) reported on 10 series with 9,119 patients: results of needle biopsy were benign in 74%, inadequate or suspicious in 22 %,and malignant in 4 % of those series. Several studies reveal that FNA biopsy sensitivity vary from 68 to 98% (mean 83%) and specificity varies from 72% to 100% (mean 92%).In many centers the post-operative surgical yield of thyroid cancer in excised thyroid nodules after pre-operative FNA biopsy has increased from about 15% to about 30%. Therefore FNA biopsy represents a tremendous advance (11).

Other background tests that can be done include radionuclide scanning to determine hot (hyper-functioning), warm (normal), and cold (hypofunctioning) nodules and ultrasonography to determine the size and number of thyroid nodules(11). Serum antithyroid peroxidase (formally called antimicrosomal) antibody and antithyroglobulin antibody levels are helpful in diagnosis of Hashimoto's disease especially if serum TSH is increased. The antibodies are positive on more than 95 % of adult patients with this disease. A baseline serum thyroglobulin is not useful or cost effective. Its value lies in serial determination after thyroidectomy following treatment of thyroid cancer. In patients with a family history of medullary carcinoma thyroid specific genetic testing and calcitonin levels should be determined. Serum antithyroid peroxidase, antithyroglobulin antibody,

thyroglobulin and calcitonin levels is neither routinely necessary or cost effective in patients with thyroid nodules (11).

Hyperthyroidism is a hypermetabolic state of the thyroid gland caused by elevated levels of T3 and T4 or isolated T3 alone. Primary hyperthyroidism arises from an intrinsic thyroid abnormality while secondary hyperthyroidism arises from processes outside the thyroid such as a TSH secreting pituitary tumour, which is rare. It is caused by a variety of disorders which includes diffuse hyperplasia (Graves disease), toxic multinodular goitre, toxic adenoma, thyroiditis (acute / subacute), hyperfunctioning thyroid carcinoma, iatrogenic (exogenous) hyperthyroidism and TSH secreting pituitary adenoma (secondary hyperthyroidism). A diagnosis of hyperthyroidism is made using clinical, and physical examination and laboratory tests. Clinical manifestations of hyperthyroidism include an increase in basic metabolic rate (BMR) and an overactivity of the sympathetic nervous system due to excess thyroid hormones T3 and T4. This includes nervousness, palpitations, rapid pulse, fatigability, muscular weakness, weight loss with good appetite, diarrhea, heat intolerance, warm skin, excessive perspiration, emotional liability, menstrual changes, a fine tremor of the hand, eye changes (ophthalmopathy) and enlargement of the thyroid gland. Laboratory findings include increased levels of T4 and T3 and decreased levels of TSH in primary hyperthyroidism(12).

Hypothyroidism is caused by inadequate levels of thyroid hormones T3 and T4. Primary hypothyroidism arises from an intrinsic abnormality in the thyroid gland while secondary hypothyroidism is a result of hypothalamic or pituitary disease. Primary hypothyroidism accounts for the majority cases, the most common being an autoimmune disease called Hashimoto's thyroiditis. Other causes are thyroid surgery (thyroidectomy) for the treatment of hyperthyroidism causing hypothyroidism and irradiation with radioiodide in the treatment of hyperthyroidism also causing hypothyroidism. Irradiation of lesions in the neck region can also cause hypothyroidism. Infiltrative diseases like hemochromatosis, amyloidosis and sarcoidosis and drugs to decrease thyroid secretions eg. methimazole and propylthiouracil can cause hypothyroidism. Secondary hypothyroidism is caused by TSH deficiency due to pituitary disease while tertiary hypothyroidism is caused by TRH deficiency due to a hypothalamic disorder. Clinical features are characterized by a slowing of physical and mental activity due to reduced BMR and decreased sympathetic activity. The classical clinical manifestations of hypothyroidism include cretinism and myxedema. Cretinism refers to hypothyroidism developing in infancy or early childhood. Clinical features

include severe mental retardation, short skeletal stature, coarse facial features, a protruding tongue and umbilical hernia. Myxedema develops in the older child or adult. Clinical features are characterized by slow physical and mental activity such as generalized fatigue, apathy, mental sluggishness, cold intolerance, and being overweight. Decreased sympathetic activity results in constipation and decreased sweating. Coarsening of facial features, enlargement of the tongue and deepening of the voice occurs (12).

Disorders of the thyroid gland come to clinical attention because of the enlargement of the gland called a goitre, oversupply and undersupply of thyroid hormones, hyperthyroidism and hypothyroidism respectively. A goitre is an enlargement of the thyroid gland which may be diffuse, multinodular or focal. The causes of thyroid gland enlargement (goitre) range from inflammation, compensatory hyperplasia and hypertrophy, autoimmune diseases and neoplasia. Inflammatory conditions include acute thyroiditis, subacute (De Quervains) thyroiditis, and Reidel's Thyroiditis. Graves disease and Hashimoto's thyroiditis are autoimmune diseases. Compensatory hyperplasia which may be endemic or sporadic are commonly known as goitres (diffuse or nodular). Cystic lesions could be due to cystic degeneration of a goiter/nodule or thyroglossal cysts. Thyroid neoplasms which can be benign or malignant include follicular neoplasm (follicular adenoma, follicular carcinoma), Hurthle cell neoplasm, papillary carcinoma, medullary carcinoma, anaplastic carcinoma, malignant lymphoma and metastatic carcinoma.

Cytological findings and hormonal profiles of thyroid nodules (goiters).

1. **Acute thyroiditis.** Acute thyroiditis is a rare condition caused by bacterial or fungal infections and is diagnosed clinically without the need for FNA. FNA cytology aspirates are composed predominantly of neutrophils and inflammatory debris and thyroid epithelial cells are scant and degenerate (13). Hormonal profile levels of TSH, T3, T4 are within reference range (euthyroid) or can be varied according to the extent of destruction.

2. **Subacute (De Quervain's) thyroiditis.** Subacute thyroiditis is also referred to as granulomatous thyroiditis, De Quervain's thyroiditis, pseudo-tuberculous thyroiditis, giant cell thyroiditis or viral thyroiditis. It is most likely of viral origin. The patients present with clinical symptoms including a tender enlarged thyroid gland. FNA cytology picture is characterized by foreign body type giant cell or giant multinucleated histiocytes. These cells have abundant granular cytoplasm with

numerous nuclei. Follicular cells are variably present and may show hurthle cell changes. The background contains inflammatory cells including neutrophils, lymphocytes and plasma cells. Due to the presence of lymphocytes and giant multinucleated cells, Hashimoto's thyroiditis and papillary carcinoma should be considered as differential diagnoses (12).

Hormonal profile levels of T3, T4, and TSH are variable. They are either euthyroid or hyperthyroid with high levels of T3 and T4 and low levels of TSH or subclinical hyperthyroidism.

3. Reidel's thyroiditis. This is a rare condition of unknown cause also referred to as fibrous thyroiditis or fibrous struma and clinically it mimics anaplastic carcinoma. The disease is characterised by extensive fibrosis of the thyroid gland, which may also involve adjacent organs. The thyroid becomes rock hard and firm. FNA cytology is difficult and yields little cellular material. Surgical biopsy is recommended. Hormonal profile levels of T3 and T4 is reduced while TSH is increased (hypothyroid).

4. Graves disease. Graves disease is an autoimmune disease associated with diffuse goiter, hyperthyroidism, ophthalmopathy and sometimes dermatopathy. It has a female preponderance of 5:1. The pathogenesis involves a defect in antigen-specific suppressor T cells with formation of autoantibodies. It is associated with HLA - DR3. These autoantibodies include antibodies to the TSH receptor (thyroid stimulating immunoglobulin TSI) which stimulate thyroid hormone synthesis of T3 and T4. Others are thyroid growth-stimulating immunoglobulins which are directed against TSH receptors causing follicular cell growth. Patients are usually young females with classical clinical manifestation of hyperthyroidism which include weight loss, muscle weakness, goiter and tachycardia. Ophthalmopathy and sometimes dermatopathy occurs. Exophthalmos is a characteristic wide staring gaze with lid lag and the eyes protrude abnormally. FNA cytology picture is rich with blood, little colloid and occasional follicular cells with enlarged nuclei. The cytoplasm is abundant with numerous vacuoles containing thin deposits of colloid. Laboratory hormonal profile findings include increased levels of T4, T3 and decreased levels of TSH. The usefulness of FNA in Graves disease is limited because the diagnosis is generally made from clinical features and laboratory data (12,13)

5. Hashimoto's thyroiditis. Hashimoto's disease is also referred to as lymphocytic thyroiditis, or chronic lymphocytic thyroiditis of autoimmune phenomena. It is an autoimmune disease. Typically

it presents with goitrous enlargement and in later stages hypothyroidism. The patient may have been euthyroid or hyperthyroid earlier. The thyroid parenchyma is virtually replaced by lymphoid infiltrate with germinal centers. The pathogenesis involves a defect in antigen-specific suppressor T cells with formation of autoantibodies. It is associated with HLA-DR5. These autoantibodies include TSH - receptor antibodies (TRA) which bind and block TSH receptor sites hence preventing synthesis of T3 and T4, antibodies to microsomes, thyroid peroxidase, thyroglobulin, T3, T4 and follicular cell membranes. The possible mechanisms for thyroid injury are antibody dependent cell cytotoxicity (ADCC) via killer cells, complement fixation and direct T cell cytotoxicity via CD8+. FNA cytologic picture is usually cellular and composed predominantly of small mature lymphocytes, plasma cells and colloid is scant or absent. There are sheets or clusters of Hurthle (oncocytic) cells that are pleomorphic with enlarged granular cytoplasm and nuclei vary in size with prominent nucleoli. Some follicular cells may be present. The differential diagnoses are malignant lymphoma (due to numerous lymphocytes), Hurthle cell neoplasm and papillary carcinomas. Laboratory findings in most cases is hypothyroid with decreased levels of T3 and T4 with compensating increase in TSH levels (15).

6. Nodular goiter. Enlargement of the thyroid or goitre is the most common manifestation of thyroid disease. Diffuse and multinodular goitre reflect compensatory hyperplasia and hypertrophy of follicular cells most commonly due to iodine deficiency. Other synonyms include colloid goitre and adenomatous goitre. This leads to impairment of T4, T3 synthesis which in turn causes hypertrophy and hyperplasia and ultimately enlargement of thyroid gland. Diffuse non-toxic (simple) goitre is a gland that is diffusely enlarged without producing nodularity. The enlarged follicles are filled with colloid hence the term colloid goiter. Epidemiologically the disorder occurs in two forms namely endemic goiter and sporadic goitre. Endemic goitres occurs in geographical areas where the soil, water and food supply contain low levels of iodine. The prevalence must be more than 10% of the general population for it to be called endemic goitre. Its prevalence has been reduced secondary to the use of iodised salt. Iodine deficiency impairs the ability of the gland to elaborate T3 and T4 hence there is compensatory increase in TSH levels inducing hypertrophy and hyperplasia of the gland. Dietary goitrogens such as excessive calcium, fluorides, thiocyanates and vegetables belonging to brassica and cruciforme families (eg. cabbages, cauliflower, cassava) may contribute to endemic goiter. Sporadic goitre is probably multifactorial in origin. Mild lack of iodine, ingestion of goitrogens like excessive calcium, thiocyanates, fluoride, foods like cabbages, cassava, drugs like lithium, phenylbutazone, and paminosalicylic acid impairs thyroid hormone

synthesis. Hereditary thyroid dyshormogenesis, autoimmune defects and physiological and pathological stresses such as puberty, infection and pregnancy may cause sporadic goitre. TSH levels are elevated in some but not all patients. Virtually all long-standing simple goiters convert to multinodular goitres. They may be nontoxic or toxic causing thyrotoxicosis. Multinodular goitre produces the most extreme thyroid enlargements and occur both in sporadic and endemic forms with higher female preponderance. The cytologic picture of both diffuse and multinodular goitres may be that of colloid goiter or adenomatous goitre. Colloid goiters are usually hypocellular aspirates containing abundant colloid, scant follicular cells, and inflammatory cells, mixed with blood. The colloid appears bluish - violet in May Grunwald Giemsa (MGG) stained smears and blue-green with Papanicolou stains. The cytologic pattern is dominated by follicular cells, which may be dispersed or grouped into follicles or small monolayers. They are sometimes described as having a honeycomb pattern though distinct cell membranes are unusual. If monolayered they are small, uniform, usually cuboidal in shape with central nuclei. The nuclei tend to be small, hyperchromatic without visible nucleoli. Naked follicular cell nuclei are often seen scattered throughout the smear that resembles mature lymphocytes. A fairly high proportion of aspirates yield cystic fluid. This fluid is sometimes clear and yellow but most commonly it is stained brown due to prior hemorrhage. Such fluid indicates degenerative cystic changes in goitre such as foamy macrophages and haemosiderin-laden macrophages. Hurthle cells can also be present in some multinodular goitres. Adenomatous goitre are usually hypercellular aspirates showing very little colloid or none at all and numerous follicular cells arranged in monolayers, microfollicles or tissue fragments. Microfollicles without or with little colloid may be seen. The nuclei are small and uniform and contain evenly distributed chromatin. Hurthle cells can also be found. Patients with multinodular goitres and non-toxic multinodular goitre are euthyroid with normal levels of T3, T4 but with increased levels of TSH while patients with toxic multinodular goitre are hyperthyroid with increased levels of T3, T4 and decreased TSH levels (16).

7. Thyroglossal duct cysts. Thyroglossal duct cysts are midline structures. These cysts are generally considered to be derived from a remnant of the thyroglossal duct, which normally disappears between the 6th - 8th week of intra-uterine life. FNA yields cell free fluid in about 25% of the cases. Cellular aspirates most often contain inflammatory cells with a mixture of squamous cells and occasionally columnar cells can be obtained from about 75% of cases. Hemosiderin-laden macrophages may sometimes be noted. Hormonal profile levels of TSH, T3, T4 are within reference range (euthyroid).

8. Follicular neoplasms. Follicular adenoma and follicular carcinoma is dealt with under follicular neoplasm as surgical removal of follicular neoplasms for final diagnosis is recommended. FNA cytology cannot classify these lesions with precision because they are defined by histologic and not cytologic criteria. Follicular adenomas are solitary benign encapsulated neoplasms. Most are "cold" nodules which show decreased RAIU by thyroid scan but in some patients toxic follicular adenomas do occur. In such patients T₄, T₃ levels are high and TSH levels are low resulting in hyperthyroidism. There are (3) three histological types of follicular adenoma. Macrofollicular (colloidal) with abundant colloid, microfollicular in which the follicles are smaller than normal and trabecular adenoma in which follicular cells are arranged in ribbons rather than spheres. Diffuse or multinodular goitres histologically are not encapsulated in contrast to follicular adenoma. FNA smear patterns of follicular adenomas resemble that of adenomatous goitres and is difficult to distinguish unless by an experienced cytopathologist hence histological evaluation is recommended in which a capsular component is evident. Patients with follicular adenoma are euthyroid while patients with toxic follicular adenoma are hyperthyroid with increased levels of T₃, T₄ and decreased TSH levels (12,13). Follicular carcinoma is the second most common malignant thyroid cancer constituting 5-15% of thyroid cancers. It is biologically more aggressive than papillary carcinoma and tends to metastasise to the lung, bones and other sites. Although the histologic spectrum can range from well-differentiated follicular growth to anaplastic growth with few follicles, the majority are well differentiated. The histology of well-differentiated encapsulated carcinoma may closely resemble follicular adenoma. The diagnosis will rest on histologic demonstration of capsular infiltration and vascular invasion. Unfortunately these are not seen in FNA specimens even though the diagnosis of follicular carcinoma depends on histologic evaluation. FNA is useful in distinguishing lesions that are benign from those that are malignant. FNA cytology smears composed of large spheres (micro follicles) and normal follicular cells with colloid are considered benign thyroid nodules by FNA while those with hypercellularity, microfollicular or trabecular patterns with overlapping cells and scant colloid are considered "suspicious" follicular nodules. Nuclear atypia in a minority of cases may be present together with nucleoli. This is the reason follicular adenoma and follicular carcinoma are classified as follicular neoplasm. Hormonal profile is usually euthyroid or hyperthyroid.

9. Hurthle cell neoplasm. Hurthle cells are transformed cells, which have abundant cytoplasm. These cells are also known as oncocytes, oxyphils or Askanazy cells. They constitute 5% of all thyroid tumours. FNA cytologic smears are often very cellular. The cells are either single or in

loose cohesive groups. The Hurthle cells are monomorphic and rarely form follicles. The cells have abundant eosinophilic cytoplasm. The nuclei are large and often binucleated. They have a prominent single nucleolus. Background colloid is scant or absent. Hurthle cell adenomas and carcinomas are difficult to distinguish by FNA. Vascular or capsular invasion is the pathologic criteria for malignancy. Hormonal profile is either euthyroid or hypothyroid(17).

10. Papillary carcinoma. Papillary carcinoma is the most common thyroid malignancy accounting for 70-85% of thyroid cancers. It usually presents as a solitary and enlarging "cold" nodule although rarely "warm" nodules are encountered. History of external neck radiation is a predisposing factor (12,18).

Given an adequate sample the FNA cytologic diagnosis is straightforward. The cytologic features include flat sheets and papillary clusters some in three dimensions. Microfollicles may be seen in the follicular variant of papillary carcinoma. The diagnosis of papillary carcinoma does not rest on morphological features alone but on nuclear changes. These include longitudinal grooves, intranuclear cytoplasmic pseudo-inclusions (holes) and pale powdery chromatin pattern showing ground-glass appearance (Orphan -Annie appearance). Nuclei are enlarged and crowded and may show molding. Nuclei are usually present and may be small or large. The N/C ratio is high. Colloid may be abundant or scant. Psammoma bodies are seen in 15-20% of the cases. Multinucleated giant cells are common. Cystic degeneration occurs in 17% of cases and can cause false negative diagnosis. The hormonal profile are either euthyroid or hyperthyroid. In "warm" nodules it may be hyperthyroid.

11. Medullary carcinoma. It accounts for 5-10% of thyroid cancers. It arises from parafollicular C cells which synthesize calcitonin. The patient's serum calcitonin levels can be used to screen this cancer. Most of medullary carcinomas occur sporadically 80-90%, but in 10-20% of cases especially in children it may be associated with neoplasms of other endocrine glands like pheochromocytoma and parathyroid hyperplasia or adenoma (MEN syndromes). FNA cytology shows mostly isolated tumor cells but may form clusters and rosettes. Nuclei are eccentrically placed giving the cells a plasmacytoid appearance and binucleation or multinucleation is common. In some cases the cells are spindle-shaped. Nuclei have salt-pepper chromatin pattern and intranuclear inclusions are quite common. Amyloid is present in most cases which can be demonstrated with Congo red stain. Hormonal profiles of TSH, T3, T4 is normal but calcitonin level is raised hence serum calcium levels is decreased (hypocalcemia).

12. Anaplastic carcinoma . Anaplastic carcinoma of the thyroid makes up 10-15 % of thyroid cancers and is one of the most aggressive thyroid neoplasms. Clinically they occur in people past the age of 60 years and grow rapidly, and by this time it has already metastasised (19). The most common symptoms are dysphagia, hoarseness and dyspnea. FNA cytology picture is quite characteristic consisting of both giant and spindle shaped cells. The cells show pleomorphism with cells exhibiting bizarre shapes and sizes. The nuclei are large with coarsely clumped chromatin, large nucleoli, intranuclear inclusions and bizarre mitotic figures. Multinucleated giant cells are also noted. The cytoplasm is dense or vacuolated. The thyroid hormonal profile expected is hypothyroidism due to destruction of follicular cells. Tumour diathesis and necrotic cells are seen.

13. Malignant lymphoma. Malignant lymphoma, particularly Non Hodgkin's lymphoma may arise in the thyroid as a primary tumour especially in the setting of Hashimotos thyroiditis. These tumors are most common in the elderly and often present as a rapidly enlarging unilateral thyroid mass, with dysphagia, hoarsness and dyspnea. The FNA cytology hallmark of Non-Hodgkin lymphoma is a uniform monomorphic population of typical lymphoid cells. Primary disease of the thyroid is extremely rare. The main differential diagnosis is Hashimotos thyroiditis. Large cell lymphomas are easiest to distinguish from Hashimotos thyroiditis unlike small cell lymphomas and mixed small and large cell lymphomas. Tumor marker studies are recommended for lymphomas. The hormonal profile may be euthyroid or hypothyroid(20,21,22).

14. Metastatic carcinoma. Metastatic carcinoma of the thyroid is extremely rare. The breast, kidney, skin, and lung are the most common primary sites. Metastatic carcinoma should be considered in patients who have a history of cancer elsewhere in the body. However, in 25-50% of cases there is no prior history of malignancy. There are usually two (2) cytologic patterns. Nodules less than 2mm in size show mixture of cancer cells and benign follicular cells while large palpable nodules show only cancer cells. Differentiation of metastatic cancer may be easy with a proper history and such feature like keratinization and mucin production. Immunocytochemistry of cell samples from FNA is useful in confirming origin of tumour. Hormonal profile may be euthyroid or hypothyroid(23,24,).

RATIONALE

Nodular thyroid disease is extremely common with a 4 – 10% lifetime risk for developing a palpable thyroid nodule. However, of these only 5% are malignant. At KNH clinicians evaluate thyroid dysfunction by measuring serum TSH, T3 and T4 which will establish euthyroidism, hyperthyroidism and hypothyroidism. In addition FNA is the diagnostic test of choice in determining whether a nodule is benign or malignant. It has not been established whether the various lesions diagnosed by FNA have differing hormonal profiles and this is the main research problem being investigated. The main aim of the study is to correlate the FNA cytologic diagnosis and hormonal profiles of patients with thyroid nodules at KNH. This information will be useful in determining whether hormonal profiles are a predictor for the various thyroidal lesions especially in differentiating benign from malignant lesions. It is expected that the results of this study will be useful in diagnosis of various lesions, as it will develop guidelines for using hormonal profiles as an adjunct to FNA cytology of thyroid nodules. It is expected that this will result in better use of resources, more accurate diagnosis and better decision making by the clinicians. This will have an added cost benefit to the patient. The investigation will also enhance better understanding of the pathology of thyroid nodules. The overall benefit of this study will be an improvement in the diagnosis and management of patients with thyroid nodules.

AIMS AND OBJECTIVES AND HYPOTHESIS

The main aim of the study was to correlate FNA cytologic diagnosis and serum TSH, T3 and T4 levels in patients with thyroid nodules seen at KNH.

Specific objectives.

The specific objectives were

1. To describe the cytological features of thyroid nodules in patients seen at KNH.
2. To determine the serum TSH, T3 and T4 levels of patients with thyroid nodules at KNH.
3. Correlate the hormonal levels to cytological findings and to make recommendations for improved diagnosis and patient management.

Null hypothesis.

There is no difference in hormonal (TSH, T4 T3) mean values in the lesions diagnosed by FNA cytology.

MATERIALS AND METHODS

1. Study Design.

This was a cross-sectional study.

2. Study Site.

Both the Thyroid clinic and FNA clinic are situated in the surgical outpatient clinic (SOPC) at Kenyatta National Hospital. They serve referral cases from peripheral hospitals and also internally referred cases from other clinics within Kenyatta National Hospital.

The thyroid clinic handles an average of 23 patients per week, which is run by consultant surgeons once per week on Monday mornings. The FNA clinic handles an average of 25 patients per week. The majority of patients requiring cytologic investigation for palpable lesions include goitres, enlarged lymph nodes, and breast lumps. An average of 8 thyroid nodules are aspirated per week. This FNA clinic is run by consultant pathologists twice per week on Monday and Thursday mornings. The specimens are then sent to the cytology laboratory for staining and reporting.

3. Study Population.

All patients who presented with enlargement of the thyroid gland and were referred to the Thyroid and FNA Clinic.

Inclusion Criteria

1. Any patient who had a nodular or diffuse thyroid goiter.
2. Any patient who had a request for FNA by the attending clinician.
3. Informed consent from patient.

Exclusion Criteria

1. Any patient who was on treatment for a thyroid disorder.
2. Refused informed consent from patient

4. (a) Sample Size.

The minimum sample size was determined using the formula $n = \frac{z^2 p(1-p)}{d^2}$ (Appendix 7)

d^2

(b) Study limitations and constraints.

Due to lack of time the number of patients studied was 42 patients. The thyroid assay kit (Serozyme ELISA kit) was also expensive and therefore only 42 patients were screened for thyroid hormones

5. Classification of Endocrine and Cytological Findings.

- a. Thyroid profile - The reference range in (Appendix 2) was used.
- b. FNA Cytology- FNA cytologic criteria (Appendix 6) was used and confirmed by the cytopathologist.

6. Data analysis.

Data was collected using a questionnaire (Appendix 2). It was then entered and analysed using Microsoft SPSS package and Analysis of Variance (ANOVA) test with a confidence limit of 95% and a 5% significance level ($p \text{ value} < 0.05$)

7. Ethical Considerations.

- a. A consent to conduct this study was obtained from the Research and Ethics committee of KNH.
- b. A consent from the patient was obtained. No adverse effects when using FNA was noted. The specimen taken and results were handled properly and confidentially respectively.
- c. Results were communicated to the attending physician as is the usual practice. All the information was treated in the strictest confidence.

METHODS OF SPECIMEN COLLECTION AND PREPARATION

1. Cytology.

After informed consent was obtained a fine needle aspiration biopsy of the thyroid gland was done (Appendix 3 {b}) and four smears (Appendix 3 {c}) for each patient was prepared by the attending pathologist and investigator at the FNA clinic. A laboratory request form was filled with the relevant details and sent to the cytology laboratory where two slides were stained with regressive Papanicolou technique and the remaining two slides were stained using standard Haematoxylin and

Eosin technique (Appendix 4). The slides were examined by the investigator and a cytological diagnosis was confirmed by the cytopathologist at the Department of Human Pathology, KNH

2. Blood Collection and Hormonal Analysis.

Approximately 5 ml of blood was withdrawn from an antecubital fossa vein and placed in a labelled plain serum bottle. Relevant information was entered into another laboratory request form. The blood was then immediately centrifuged at 2500rpm for 5 minutes and the serum obtained was stored at 20°C in a fridge at the Department of Clinical Chemistry. The serum collected over a period of time from patients was assayed once at the end of the study using serozyme ELISA technique at the Department of Clinical Chemistry, University of Nairobi. (Appendix 5).

RESULTS:

During the study period a total of 42 patients were seen at the thyroid and FNA Cytology clinic KNH. The majority of the patients were in the age group 31-40 and 41-50 years as shown in Figure 1.

Figure 1: Frequency bar graph for *age* distribution.

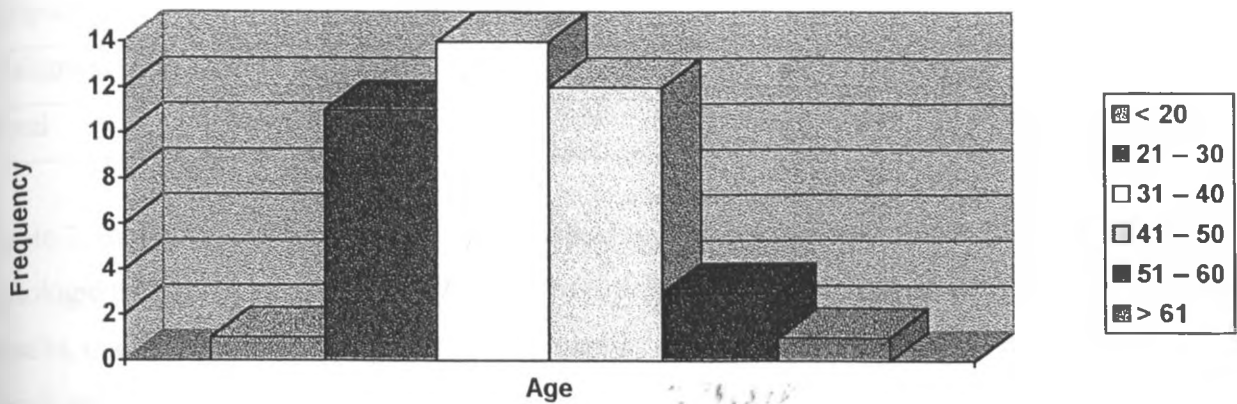


Table 1: Complete distribution table for the variable *sex*.

Sex	Frequency	% Relative frequency
Female	37	88
Male	5	11.9
Total	42	100

Ratio female: male 7 : 1

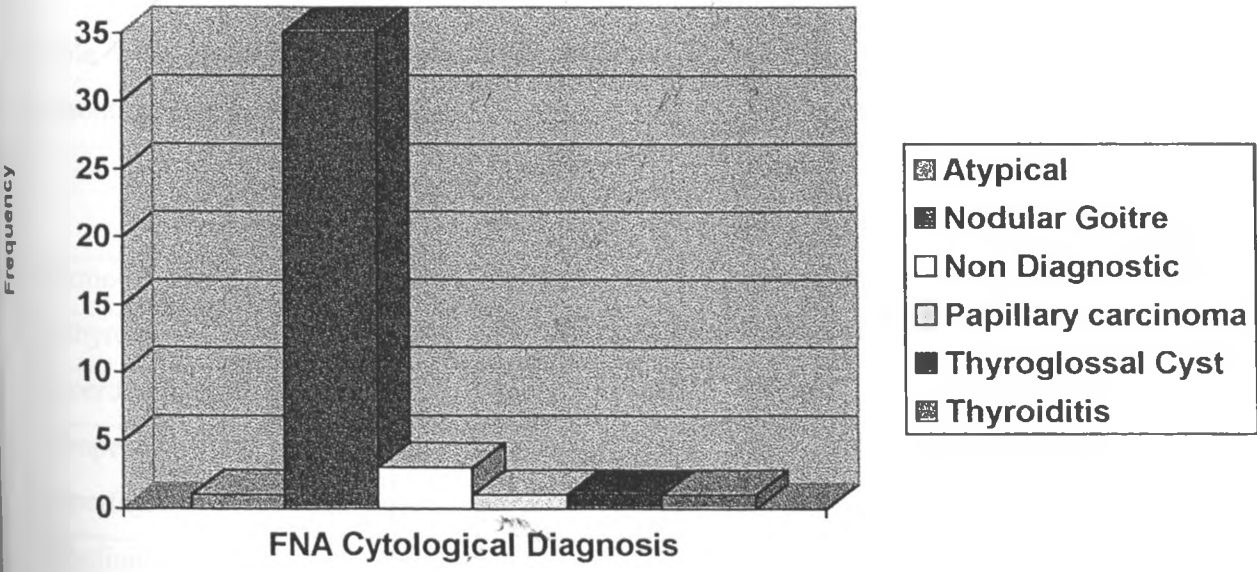
Table 1 shows that 37 (88.09%) of the patients were females, and 5 (11.90%) were males giving a female to male ratio of 7 : 1.

Table 2: Distribution table of *benign, suspicious/non – diagnostic and malignant* FNA cytologic diagnoses.

FNA Cytological Diagnosis	Frequency	%Relative frequency
Benign	37	88
Non-diagnostic	3	7.1
Suspicious	1	2.3
Malignant	1	2.3
Total	42	100

Table 2. shows the distribution of benign, suspicious, non – diagnostic and malignant FNA cytologic diagnoses. In general 37 (88%) patients had negative (benign) FNA cytologic diagnostic results, one 1 (2.3%) had a positive result of papillary carcinoma, one 1 (2.3%) had a suspicious result and 3 (7.1%) were non-diagnostic as shown in Table 2 .

Figure 2: Frequency bar graph for *FNA cytologic diagnostic groups*.



The distribution of the patients according to FNA cytological diagnosis is illustrated in Figure 2. Patients with nodular goitre comprised 35(83.3%) , non-diagnostic samples 3(7.14%), papillary carcinoma, atypia, thyroglossal cyst and thyroiditis 1(2.38%) each . No patients had a cytological diagnosis of Hashimotos thyroiditis,subacute glaucomatous thyroiditis, follicular carcinoma, medullary carcinoma, anaplastic carcinoma, malignant lymphoma and metastatic carcinoma.

Table 3 : Overall descriptive statistics of T3 (nmol/l),T4 (nmol/l),TSH (uIU/ml).

	No	minimum	maximum	mean	mean	std.
		value	value	value	std. error	deviation
T4nmol/l	42	71.4	320	135.17	8.08	52.39
T3nmol/l	42	1.23	6.2	2.46	0.15	0.96
TSHuIU/ml	42	<0.05	15	1.88	0.37	2.41

Reference range: T4 – (67- 163 nmol/l), T3 - (1.12 –2.66 nmol/l), TSH – (0.6 – 4.5 uIU/ml).

Table 3. shows the overall mean, standard deviation, minimum and maximum (range), standard error of the mean (CI of 95%) of serum T4,T3, and TSH of all the patients. The minimum value of serum total T4, T3, and TSH, was 71.4nmol/l, 1.23nmol/l, and <0.05uIU/ml respectively while the maximum value of serum total T4, T3, and TSH, was 320nmol/l, 6.2nmol/l, and 15uIU/ml respectively. The mean value of serum total T4, T3, and TSH, was 135.17nmol/l, 2.46nmol/l, and 1.88uIU/ml respectively. The overall mean favoured a diagnosis of euthyroidism.

Table 4: Hormonal profile and descriptive statistics T4(nmol/l),T3(nmol/l),TSH(uIU/l) of patients presenting with Thyroidal nodules:

Hormonal profile	No	T4nmol/l		T3nmol/l		TSHuIU/ml		%
		mean	sd	mean	sd	mean	sd	
Euthyroid	26	108.365	25.83	1.99	0.49	0.806	1.158	61.6
Hyperthyroidism	5	214.4	60.61	3.65	1.47	0.106	0.082	11.9
Compensated Hyperthyroidism	8	183.25	33.63	3.45	0.53	2.135	1.096	19
Subclinical hyperthyroidism	2	90.75	25.81	1.87	0.10	<0.05	0	4.76
Subclinical hypothyroidism	1	141	0	2.2	0	15	0	2.3
Total	42							

The statistical means and standard deviations of T4,T3,TSH of patients with euthyroidism, hyperthyroidism, compensated hypertyroidism , subclinical hypothyroidism and subclinical

hyperthyroidism are shown in Table 4. In general 26 (61.6%) had a hormonal profile of euthyroidism, 5 (11.5%) had hyperthyroidism, 8 (19%) had compensated hyperthyroidism, 2 (4.76%) had subclinical hyperthyroidism and 1 (2.3%) had subclinical hypothyroidism.

Table 5a: Hormonal profile and descriptive statistics T4(nmol/l),T3(nmol/l),TSH(uIU/l) of patients with nodular goiter.

FNA Cytology	No	T4n mol/l		T3n mol/l		TSH uIU/ml		Hormonal Profile	%
		mean	sd	mean	sd	mean	sd		
Nodular Goiter	20	108.5	27.18	2.07	0.52	1.47	0.91	Euthyroid	57
Nodular Goiter	5	214.4	60.61	3.65	1.47	0.11	0.08	Hyperthyroid	14.3
Nodular Goiter	7	178.1	32.8	3.38	0.54	2.18	1.17	Comp. Hyperthyroid	20
Nodular Goiter	1	141	-	2.2	-	15	-	Subclinical hypothyroidism	2.8
Nodular Goiter	2	90.75	25.81	1.87	0.10	<0.05	-	Subclinical hyperthyroid	5.7
Total	35								
Overall mean		137.5	53.75	2.54	0.97	1.72	2.55	Euthyroid	100

Table 5a which represents patients with nodular goiter only, shows their hormonal profile, their relative frequency and statistical mean and standard deviation of serum T4 T3 and TSH concentration. Generally 20(57%) of the patients had a hormonal profile of euthyroidism, 7 (20%) had compensated hyperthyroidism, (14.3%) had hyperthyroidism, 2 (5.7%) had subclinical hyperthyroidism and 1(2.8%) had subclinical hypothyroidism.

Table 5b: Hormonal profile and descriptive statistics of T4(nmol/l),T3(nmol/l),TSH(uIU/ml) of patients with other FNA cytology.

FNA Cytology	No	T4n mol/l		T3nmol/l		TSHu IU/ml		Hormonal profile	%
		mean	sd	mean	sd	mean	sd		
Non – diag	3	87.16	5.0	1.64	0.44	2.46	1.86	Euthyroid	7.14
Thyro – cyst	1	129	-	1.85	-	3.4	-	Euthyroid	2.38
Atypia	1	128	-	1.7	-	4.3	-	Euthyroid	2.38
Papillary. Ca	1	129	-	2.0	-	1.9	-	Euthyroid	2.38
Thyroiditis	1	219	-	3.93	-	1.8	-	Compensated hyperthyroid	2.38

The FNA cytologic diagnosis, their relative frequency, their respective statistical mean and standard deviation of serum T4 T3 TSH concentration and hormonal profile of the remaining patients is shown in Table 5b. Three (7.14 %) patients with FNA diagnosis of nondiagnostic sample had euthyroidism while 1(2.38%) patient each with papillary carcinoma, thyroglossal cyst, and atypia, had a hormonal profile of euthyroidism. One patient with thyroiditis had a biochemical diagnosis of compensated hyperthyroidism.

Table 6 : Analysis of variance (ANOVA) to determine whether serum T4(nmol/l),T3(nmol/l),TSH(uIU/ml) levels correlate with the FNA cytological diagnostic groups.

	FNA Diagnosis(Groups)	Sum of Squares	Df	Mean Square	F	Significance
T4(nmol/l)	Between Groups	14273.06	5	2854.61	1.04	0.406
	Within Groups	98285.48	36	2730.15		
	Total	112558.6	41			
T3(nmol/l)	Between Groups	5.61	5	1.12	1.23	0.311
	Within Groups	32.59	36	0.9		
	Total	38.2	41			
TSH(uIU/l)	Between Groups	10.05	5	2.01	0.31	0.9
	Within Groups	228.76	36	6.35		
	Total	238.81	41			

One way analysis of variance (ANOVA) is used to test hypotheses about population means. It is used to test the null hypothesis that there is no difference in hormonal (TSH, T3,T4) means among (between) and within lesions diagnosed by FNA (here referred to as groups). One way analysis of variance (ANOVA) with confidence limit (CL) of 95% and $p < 0.05$ was done and generally there was no significant statistical difference of the mean levels of T4($p=0.406$), T3 ($p=0.311$) and TSH ($p=0.90$) between and within the various categories of FNA cytological diagnosis as shown in Table 6.

DISCUSSION

Nodular thyroid disease has generated interest in the diagnostic methods available (25). In general these methods involve distinguishing benign from malignant thyroid nodules and determining the presence of hyperthyroidism, euthyroidism and hypothyroidism. These diagnostic methods should be both cost effective and provide quality care to the patient. Thyroidectomy whether total or partial for the diagnosis of a thyroid nodule has never been satisfactory because it requires hospitalisation and is costly. Many diagnostic methods such as thyroid function tests, radionuclide scanning, ultrasonography and FNA biopsy can be ordered for evaluation of thyroid nodules, but their application in all patients with nodules would also be a burden to the health care delivery system. Not all diagnostic methods are necessary or appropriate and for some patients only a limited evaluation is needed(25,26). This study was carried out to evaluate two diagnostic methods, FNA biopsy and the thyroïdal hormonal profile (serum total T4, T3, and TSH levels) and to assess the correlation between these two investigations.

During the study period a total of 42 apparently euthyroid patients were seen. The majority of the patients were in the age group 31 – 41 (33.3%) and 41 – 50 (28.5%) years. There were 37 females and 5 males giving a female to male ratio of 7 : 1. The high female to male ratio in the study supports the high prevalence of goiter in Kenya which is greater than 40% of the population as noted in studies carried out in East Africa.(27).

In this study 88% of the cases were benign, 7.1% of the cases were non-diagnostic, and 2.3% of the cases each were suspicious and malignant respectively. These statistics compare well with other studies carried out by Gharib et al.(28), who evaluated 7 series with a total of 18,183 FNA :69% were benign, 27% were suspicious or non-diagnostic, and only 4% were malignant. In another study by Schmidt et al (29), 72.1% cases were benign, 17.1% cases were non-diagnostic, 7.2% cases were suspicious, and 3.5% cases were malignant. Yet in another study Goellner et al (30), reported 89% cases were negative (benign), 6.8% cases were non-diagnostic, 3.5% cases were suspicious, and 0.7% cases were positive (malignant). Several studies have indicated that 70%-98% of patients with non-diagnostic FNA biopsies had benign lesions, with a low incidence of neoplasia 5%-10% after histopathological diagnosis (31,32).

The most common FNA cytological diagnostic result was nodular goiter (83.33%) followed by non-diagnostic samples (7.14%) and atypia, thyroglossal cyst, thyroiditis, papillary carcinoma (1%)

each respectively. Of 1853 thyroid lesions subjected to FNA sampling Jayaram *et al* (33) found that nodular goiter was the most common thyroid lesion sampled. Over a three (3) year period at the FNA clinic (KNH), out of 266 patients seen, (71%) of the cases were reported as nodular goitre (colloid or adenomatous), non-diagnostic (19%), atypia (1.5%) oncocytoma (0.75%), thyroiditis, follicular neoplasm and thyroid carcinoma (0.38%) each (34). This is almost comparable to this study and the slight variation could be due to the small sample size. Equally it is known the FNA procedure is deceptively simple but it takes considerable skill and experience to master the technique. The best results are obtained when a single physician (the cytopathologist) performs the biopsy and interprets the smear. Only cellular smears should be considered diagnostic and inadequate smears should not be evaluated for a diagnosis. A repeat should be recommended. FNA is generally highly sensitive (to presence of disease) and highly specific (to absence of disease)(35). Various studies show that specificity ranges between 67% to 100% and sensitivity ranges between 78% to 88% (36,37).

A sensitive biochemical analysis of serum total T₃, T₄ and TSH was done to determine thyroid function. The overall total T₄ mean serum level was 135.17 nmol/l (SD ± 8.08), for total T₃ the mean was 2.46 nmol/l (SD ± 0.15) and for TSH it was 1.88 uIU/ml (SD ± 0.37). This fell between the reference range of T₄ (67 – 163 nmol/l), T₃ (1.12 – 2.66 nmol/l) and TSH (0.6 – 4.5 uIU/ml) respectively. The hormonal profile after biochemical analysis showed that 26 (61.6%) patients were euthyroid, 5 (11.5%) were hyperthyroid, 8 (19%) had increased levels of total T₃ and T₄ but normal TSH (Compensated hyperthyroidism), 2 (4.76%) patients had subclinical hyperthyroidism, and one (2.3%) had subclinical hypothyroidism.

Most euthyroid patients with nodular goiter have serum TSH concentration in the normal range. However some of these patients may have increased levels of total T₄ and T₃ (Compensated hyperthyroidism). In our study 8 patients had normal levels of TSH associated with euthyroidism which was concordant with clinical findings but had increased levels of total T₄ and T₃ (Compensated hyperthyroidism). All had nodular goitre and one patient had thyroiditis. Therefore some of these patients but not necessarily all could be having intrinsically normal values of free T₄ and T₃. These patients need not be treated but are difficult to diagnose. This could be attributed to factors that influence serum thyroid hormone concentrations. Both T₄ and T₃ circulate in association with plasma proteins of which the most important is TBG. TBG has a higher affinity for T₄ than it does for T₃. T₄ levels may be elevated when the TBG concentration is increased as in

pregnancy, hepatitis, congenital TBG elevation and administration of estrogen in post- menopausal women or in oral contraceptives. This will give a false diagnosis of hyperthyroidism. However free T4 and T3 levels are normal. Conversely total T3 and T4 may be reduced when TBG concentration is decreased by such conditions as nephrosis, hepatic failure, congenital TBG deficiency, administration of androgens or large amounts of glucocorticoids. Some drugs like fenclofenac and salicylates compete with T4 for TBG binding sites.(38,39,40).

In the study five (5) patients had a hormonal profile of hyperthyroidism but did not show classical symptoms of hyperthyroidism. All had nodular goiter. This could be attributed to autonomous functioning thyroid nodules AFTNs (TSH-independent) that is known to occur in toxic multinodular goiter and toxic adenoma or Graves disease. Autonomous functioning thyroid nodules AFTNs may be solitary in otherwise normal glands or they may appear as single or multiple nodules in pre-existing goiter. Depending on the available iodine supply, age of the patients, duration of the lesion, and the mass of hyperfunctioning tissue , the patient may be euthyroid or hyperthyroid. Autonomous functioning thyroid nodules AFTNs are accompanied by a euthyroid clinical picture in 80% of the cases, and only 20% of the cases exhibit hyperthyroidism. Thyroid function ranges from minimal increases in serum total T3 and T4, to moderate increases of total T3 and T4, without symptoms of hypermetabolism, to marked increases in thyroid function with severe clinical symptoms. TSH is usually normal, but can progressively be suppressed by T3 and T4 levels. In Graves disease, occasionally thyroid enlargement rather than symptoms of hyperthyroidism is the presenting complaint but the later usually is evident on questioning. All patients with toxic autonomous thyroid nodules require treatment.

Although most patients with hyperthyroidism usually have overt clinical and biochemical disease, hyperthyroidism may be subclinical. Many patients with AFTNs (TSH-independent) have subclinical hyperthyroidism. This is most often defined biochemically as normal serum T4, and T3 and decreased TSH concentration. Patient's should be treated at the time of diagnosis. Two (2) patients were classified as such. These patients may or may not have symptoms or signs of hyperthyroidism and if present the symptoms are usually mild. Older patients should be treated because of cardiac implications. Subclinical hypothyroidism can be defined as an asymptomatic state associated with normal total T4,T3 and an elevated serum TSH concentration. In our study one patient had a high level of TSH (15uIU/ml) and the total serum T4,T3 levels were within reference range. Cytologically all these patients had nodular goitres. This is expected although not

all patients with subclinical hyperthyroidism or subclinical hypothyroidism have nodular goitres.(41,42).

Approximately 80% of patients had FNA cytological diagnosis of nodular goiter and this corresponded with a hormonal profile of euthyroidism (57%) followed by compensated hyperthyroidism (20%), hyperthyroidism (14.3%), subclinical hyperthyroidism (5.7%) and subclinical hypothyroidism (2.8%) respectively. This shows that there is no correlation between nodular goitre and serum total T4, T3 and TSH concentration. Patients with diffuse goiter due to Graves disease, simple or multinodular goiter and follicular adenoma (non-toxic or toxic) usually have the same cytopathological FNA diagnosis(43). One patient had non-specific thyroiditis with increased total serum T4,T3 concentration. Studies have indicated that thyroid function could range from subclinical hyperthyroidism to overt hyperthyroidism in the acute phase. This is due to disruption of thyroid follicles and release of thyroid hormones into the circulation. As the thyroiditis subsides serum thyroid hormones levels fall to normal(43).

The patients who were diagnosed cytologically with thyroglossal cyst, atypia, papillary carcinoma and non-diagnostic specimens had a biochemical diagnosis of euthyroidism. Other studies have indicated that patients with thyroglossal cysts have normal serum T3,T4 and TSH concentration. A cystic nodule could also exist within a nodular goiter.

Studies have also indicated that only rarely do patients with thyroid nodules that are malignant have hyperthyroidism or hypothyroidism. In this study only one patient had a malignancy (papillary carcinoma) but had normal serum levels of total T4, T3 and TSH concentrations (euthyroid). Alagol et al (44) reported three 3 patients with lymphoma of the thyroid and one case of anaplastic thyroid carcinoma with hyperthyroidism. An abnormal TSH,T3,T4 determination decreases the suspicion but does not eliminate the possibility of malignancy in a thyroid nodule.(45).

One way ANOVA (analysis of variance) was done. The goal was to determine whether serum total T4,T3 and TSH concentrations vary across the six (6) FNA cytological groups seen namely nodular goitre, thyroglossal cyst, non-diagnostic, thyroidism, atypia and papillary carcinoma and also whether it varies within the groups. With a confidence interval of 95% and $p < 0.05$ there was no significant statistical difference ($p < 0.05$) of the mean levels of T4 ($p = 0.406$), T3 ($p = 0.311$) and TSH ($p = 0.90$) between and within the various groups of FNA cytological diagnoses. In this study,

no case of Hashimotos thyroiditis , follicular carcinoma, medullary carcinoma, anaplastic carcinoma, malignant lymphoma, metastatic carcinoma, subacute granulomatous thyroiditis and lymphocytic thyroiditis was encountered.

The study showed that FNA cytologic diagnosis cannot be used to predict thyroid function using total serum T4,T3 and TSH concentrations. The cytological picture of nodular goiter occurs mostly in euthyroid states due to impaired thyroid hormone synthesis because of iodine deficiency, goitrogens, and dysmorphogenesis, and also in hyperfunctional thyroid states like diffuse goiters due to Graves disease, toxic multinodular goiter, and toxic adenoma. Histology which is the 'gold standard' in the diagnosis of the thyroid nodule, together with cytology, and radionuclide scanning would be more sensitive in predicting thyroid function. Measurement of TSH, FT4, and FT3 would be preferable.

RECOMMENDATIONS

- 1 All patients with enlarged thyroids should have biochemical analysis to determine thyroid function. Measurement of TSH, FT4, and FT3 is recommended since it gives an accurate diagnosis of thyroid function.
- 2 All patients with enlarged thyroids should have FNA done with a view to excluding malignancy because it is simple, safe, cost-effective, and allows accurate cytological diagnosis of benign and malignant lesions. It will help select patients who require surgery.
- 3 Skilful sampling of the lesion should be reinforced to get a better sample and hence cytological report. This will reduce diagnostic errors such as inadequate material for diagnosis, sampling errors, and cytodiagnostic errors. This calls for increased training of pathologists, cytologists, and laboratory personnel.
4. There is need to conduct a comprehensive study at KNH to correlate hormonal profile with histology, cytology, and radionuclide scanning. A suitable study design would be a prospective (cohort) study where time, resources, personnel and adequate sample size is considered.

APPENDIX 1

CONSENT FROM PATIENT

I, _____
of _____ hereby agree to be enrolled in the study by Dr. C.
K. Sang to give blood samples for laboratory analysis, the procedure and purpose of which has
been explained and clearly understood by me. I also agree to the procedure of fine needle
aspiration biopsy, which has been explained to me.

I understand that any results that would help in the management of my condition shall be
communicated to the relevant health personnel, and me in strict confidence. It has been explained
to me that I can withdraw from the study at any time without forfeiting any health benefits to which
I am entitled.

Date: _____

Signed: _____

Witness: _____

I confirm that I have explained in full the nature of the study to the above named patient.

Signed: _____

Date: _____

APPENDIX 6

CRITERIA FOR FNA CYTOLOGIC DIAGNOSIS USED FOR THE FOLLOWING

a) Nodular Goiter.

- hypocellular aspirant contains abundant colloid, scant follicular cells, and inflammatory cells, mixed with blood
- colloid appears pink or blue-green with Papanicolou stains.
- follicular cells, may be dispersed or grouped into follicles or small monolayers with a honeycomb pattern.
- nuclei tend to be small, hyperchromatic without visible nucleoli.
- naked follicular cell nuclei are often seen scattered throughout the smear that resembles mature lymphocytes.
- cystic fluid with hemorrhage- degenerative cystic changes in goitre such as foamy macrophages and haemosiderin-laden macrophages.
- hypercellular aspirate shows very little colloid or none at all and numerous follicular cells arranged in monolayers, microfollicles or tissue fragments. Microfollicles without or with little colloid may be seen.

b) Subacute(De Quervain's)thyroiditis.

- foreign body type giant cell or giant multinucleated histiocytes-these cells have abundant granular cytoplasm with numerous nuclei.
- follicular cells are variably present and may show Hurthle cell changes.
- inflammatory cells including neutrophils, lymphocytes and plasma cells.

c) Hashimotos thyroiditis.

- predominance of small mature lymphocytes and plasma cells
- colloid is scant or absent
- sheets or clusters of Hurthle (oncocyctic) cells that are pleomorphic with enlarged granular cytoplasm.
- nuclei vary in size with prominent nucleoli.
- follicular cells may be present.

The differential diagnoses are:

Malignant lymphoma (due to numerous lymphocytes), Hurthle cell neoplasm and papillary carcinoma.

d) Thyroglossal duct cysts.

- cell free fluid in about 25% of the cases

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-cellular aspirate most often contains inflammatory cells with a mixture of squamous cells

-columnar cells can be obtained from about 75% of cases.

-hemosiderin-laden macrophages may sometimes be noted.

e) Follicular adenomas.

-resemble that of nodular goitres (adenomatous) and is difficult to distinguish unless by an experienced cytopathologist hence histological evaluation is recommended in which a capsular component is evident.

f) Follicular carcinoma.

-large spheres (micro follicles) and normal follicular cells with colloid are considered benign thyroid nodules by FNA.

-those with hypercellularity, microfollicular or trabecular patterns with overlapping cells and scant colloid are considered "suspicious" follicular nodules.

-nuclear atypia in a minority of cases may be present together with nucleoli.

-this is the reason follicular adenoma and follicular carcinoma are classified as follicular neoplasm.

g) Papillary carcinoma.

-papillary clusters and flat sheets some in three dimensions.

-microfollicles may be seen in the follicular variant of papillary carcinoma.

-nuclear changes-these include longitudinal grooves, intra nuclear cytoplasmic pseudo-inclusions (holes) and pale powdery chromatin pattern showing ground-glass appearance (Orphan -Annie appearance).

-nuclei are enlarged and crowded and may show molding-the N/C ratio is high.

-nucleoli are usually present and may be small or large

-colloid may be abundant or scant.

-psammoma bodies are seen in 15-20% of the cases.

-multinucleated giant cells are common.

-cystic degeneration occurs in 17% of cases and can cause false negative diagnosis.

h) Medullary carcinoma.

isolated tumor cells but may form clusters and rosettes.

-nuclei are eccentrically placed giving the cells a plasmacytoid appearance and binucleation or multinucleation is common.

Amyloid is present in most cases which can be demonstrated with Congo red stain.

i) Anaplastic carcinoma.

-giant and spindle shaped cells which show pleomorphism with cells exhibiting bizarre shapes and sizes.

-nuclei are large with coarsely clumped chromatin, large nucleoli, intranuclear inclusions and bizarre mitotic figures.

-multinucleated giant cells are also noted.

j) Malignant lymphoma.

-Non-Hodgkin lymphoma is a uniform monomorphic population of typical lymphoid cells. Primary disease of the thyroid is extremely rare.

-Tumor marker studies are recommended for lymphomas.

k) Metastatic carcinoma.

-two (2) cytologic patterns.

-nodules less than 2mm in size show mixture of cancer cells and benign follicular cells while

-large palpable nodules show only cancer cells.

-Immunocytochemistry of cell samples from FNA is useful in confirming origin of tumour.

APPENDIX 7

Determination of sample size

The minimum sample size (n) is determined using the formula

$$n = \frac{z^2 p(1-p)}{d^2}$$

z = reliability coefficient

p = prevalence of a condition in a population.

d = absolute precision (difference between mean or p and the upper and lower limit).

Since

Reliability coefficient is 1.96 (confidence interval of 95 %) and

p is 90% which is the prevalence of patients with goiter who have a normal hormonal profile.

d = 0.1

Therefore
$$n = \frac{(1.96)^2 0.9(1-0.90)}{0.1^2} = 34.5$$

The minimum sample size (n) is 35, this means the sample size should be greater than 35

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Ref. KNH-ERC/01/1320.

Date.....4 April 2002.....

Dr. Conway K. Sang
Dept. of Pathology
Faculty of Medicine
University of Nairobi

Dear Dr. Sang,

RE: RESEARCH PROPOSAL "CORRELATION BETWEEN CYTOLOGIC DIAGNOSIS AND SERUM
THYROTROPHIN AND THYROID HORMONE MEASUREMENTS IN PATIENTS WITH THYROID
NODULES" (P36/4/2001)

This is to inform you that the Kenyatta National Hospital Ethical 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 data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Thank you.

Yours faithfully,

A handwritten signature in cursive script, appearing to read 'A. Guantai', is written over the typed name.

PROF. A.N. GUANTAI
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