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CORRELATION BETWEEN CYTOLOGY AND THYROID FUNCTION TEST
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ABSTRACT

Background: **Thyroid dysfunction can be evaluated by measuring serum thyroid stimulating hormone (TSH), total tri-iodothyronine (T3) and total thyroxine (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.**
Objective: **To correlate hormonal levels to FNA cytologic findings.**
Design: **A cross-sectional study.**
Setting: **Kenyatta National Hospital (KNH).**
Results: **Forty two patients had their thyroid profiles done and the results were correlated with FNA diagnosis. Majority of patients had nodular goiter (83.3%), of which 47.6% had euthyroidism, 16.7% had biochemical euthyroidism, 11.9% had hyperthyroidism, 4.8% had sub-clinical hyperthyroidism and 2.4% had sub-clinical hypothyroidism. Three patients (7.1%) with FNA diagnosis of non-diagnostic sample had euthyroidism while 2.4% each with papillary carcinoma, thyroglossal cyst, and atypia, had a hormonal profile of euthyroidism. There was no significant statistical difference ($p>0.05$) of the mean levels of T4 (0.406), T3 (0.311), and TSH (0.90), between and within the various groups of FNA cytological diagnoses.**
Conclusion: **The study showed that there was no correlation between T4, T3, and TSH measurements and FNA cytological diagnoses.**

INTRODUCTION

The basic morphologic unit of the thyroid gland is the follicle, composed of follicular cells which produce the hormones tri-iodothyronine T3 and thyroxine T4, which are in turn regulated by thyroid stimulating hormone (TSH), produced by the anterior pituitary gland. Diseases of the thyroid gland usually result in thyroid enlargement (goiter) which can be due to infections, cystic changes, autoimmune diseases, and iodine deficiency leading to hyperplasia and neoplasia, with or without thyroid hyperfunction (hyperthyroidism) or thyroid hormone deficiency (hypothyroidism). There have been many surveys of the prevalence of goitre in many countries (1). It is not uncommon to find 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 infact malignant (2).

The diagnosis of hyperthyroidism and hypothyroidism can be made using different methods but the most common ones are serum TSH levels in conjunction with total and free T4 and T3

levels. Rarely do patients with malignant thyroid nodules have hyperthyroidism or hypothyroidism (3). Age -related variations, illness, pregnancy nutritional changes and various medications may cause normal reference ranges to change. The arguments for measurements of TSH, T3 and T4 is strongest before treatment is started (4,5). 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. 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 (6).

At Kenyatta National Hospital (KNH), clinicians usually 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.

MATERIALS AND METHODS

Study design and site: This was a cross-sectional study of forty two patients seen at the thyroid and FNA clinic at KNH between June and August 2001.

Inclusion and exclusion criteria. Patients were included if they had a nodular or diffuse thyroid goiter, and had a request for FNA by the attending clinician. Any patient who was on treatment for a thyroid disorder was excluded.

Ethical considerations: Ethical approval was obtained from the research and ethics committee of KNH and informed consent from the patient before data collection.

FNA Cytology: Fine needle aspiration biopsy of the thyroid gland was done and four smears for each patient was prepared 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. The slides were examined and a cytological diagnosis was confirmed by the cytopathologist at the Department of Human Pathology, KNH.

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 2500 rpm for five minutes and the serum obtained was stored at 20ºC in a freezer until the time of analysis 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 ELISA kits from Human Gesellschaft fur Biochemica und Diagnostic MbH Max-Planck-Ring 21, D-6205 Wiesbaden, Germany Serozyme ELISA. The reference range used was T4 -(67-163 nmol/l), T3 -(1.12-2.66 nmol/l) and TSH -(0.6 - 4.5 uIU/ml).

Data analysis: Data were analysed using SPSS and an Analysis of Variance (ANOVA) test with a confidence limit of 95% and a *5%* significance level (p-value<0.05).

RESULTS

A total of 42 patients were seen at the thyroid and FNA clinic at KNH. Nodular goitre comprised 83.3%, non-diagnostic samples 7.1%, with papillary carcinoma, atypia, thyroglossal cyst and thyroiditis 2.4% each (Table 1). No patients had a cytological diagnosis of Hashimotos thyroiditsis, subacute thyroiditis, follicular carcinoma, medullary carcinoma, anaplastic carcinoma, malignant lymphoma and metastatic carcinoma. Majority of patients had nodular goiter of which 47.6% had euthyroidism, 16.7% had biochemical euthyroidism which is defined as normal levels of TSH associated with elevated levels of T4 and T3 above reference range, 11.9% had hyperthyroidism, 4.8% had sub-clinical hyperthyroidism and 2.4% had sub-clinical hypothyroidism (Table 2).

Table 1

Distribution for FNA cytology (n = 42)

FNA cytology	% Relative frequency
Nodular goitre	83.3
Non-diagnostic	7.1
Papillary carcinoma	2.4
Atypia	2.4
Thyroglossal cyst	2.4
Thyroiditis	2.4
Total	100

Table 2

Hormonal profile and descriptive statistics of TSH,T4, T3 and FNA cytology groups (n = 42)

FNA cytology	Hormonal profile	(%)	T4nmol/l Mean ± SD	T3nmol/l Mean ± SD	TSHuIU/ml Mean ± SD
Nodular goiter	Euthyroidism	47.6	108.50 ± 27.18	2.07 ± 0.52	1.47 ±0.91
	Hyperthyroidism	11.9	214.40 ± 60.61	3.65 ± 1.47	0.11 ±0.08
	Biochemical euthyroidism	16.7	178.10 ± 32.80	3.38 ± 0.54	2.18 ± 1.17
	Subclinical hypothyroidism	2.4	141.00	2.20	15.00
	Subclinical hyperthyroidism	4.8	90.75 ±25.81	1.87 ± 0.11	<0.05
Non–diagnostic	Euthyroidism	7.1	87.10 ± 5.00	1.64 ± 0.44	2.46 ± 1.86
Thyroglossal cyst	Euthyroidism	2.4	129.00	1.85	3.40
Atypia	Euthyroidism	2.4	128.00	1.70	4.30
Papillary carcinoma	Euthyroidism	2.4	129.00	2.00	1.90
Thyroiditis	Biochemical euthyroidism	2.4	219.00	3.93	1.80
Total		100			

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

It was noted that 7.1 % of patients with non–diagnostic sample had euthyroidism while 2.4% each of patients with papillary carcinoma, thyroglossal cyst, and atypia, had a hormonal profile of euthyroidism. One patient with thyroiditis had a biochemical diagnosis of biochemical euthyroidism. One way analysis of variance (ANOVA) is used to test the null hypothesis that there is no difference in hormonal (TSH,T3,T4) means 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 ($p>0.05$) of the mean levels of T4 (0.406), T3 (0.311) and TSH (0.90) between and within the various categories of FNA cytological diagnosis (Table 3).

Table 3

Correlation of serum T4 (nmol/l), T3(nmol/l), TSH(uIU/ml) levels and the FNA cytological diagnostic groups

	FNA diagnosis (Groups)	Df	Mean square	F	Significance
T4(nmol/l)	Between groups	5	2854.61	1.04	0.406
	Within groups	36	2730.15	–	–
	Total	41			
T3(nmol/l)	Between Groups	5	1.12	1.23	0.311
	Within Groups	36	0	0.90	–
	Total	41			
TSH(uIU/l)	Between Groups	5	2.01	0.31	0.9
	Within Groups	36	6.35	–	–
	Total	41			

DISCUSSION

The most common FNA cytological diagnostic result was nodular goiter (83.3%) followed by non-diagnostic samples (7.1%). Atypia, thyroglossal cyst, thyroiditis, and papillary carcinoma accounted for (2.4%) each. Of 1853 thyroid lesions subjected to FNA sampling Jayaram *et al* (7) found that nodular goiter was the most common thyroid lesion sampled. Over a three 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(8). This is almost comparable to this study and the slight variation could be due to the small sample size.

In this study, 19.1% of patients had normal levels of TSH associated with euthyroidism which was concordant with clinical findings but had increased levels of total T4 and T3 (Biochemical euthyroidism). This is attributed to thyroxine binding globulin TBG, which may lead to anomalous results. T4 levels may be elevated when the TBG concentration is increased as in pregnancy, hepatitis, congenital TBG excess and administration of oestrogen or oral contraceptives. 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, glucocorticoids, and some drugs like fenclofenac and salicylates (9-11). TBG levels were not estimated in this study.

It is worth noting that 11.9% patients with nodular goiter had a hormonal profile of hyperthyroidism but did not show classical symptoms of hyperthyroidism. This could be attributed to autonomous functioning thyroid nodules AFTNs (TSH-independent). Autonomous functioning thyroid nodules AFTNs are accompanied by a euthyroid clinical picture in 80% of the cases, 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 (12).

Subclinical hypothyroidism is usually diagnosed during routine examination and is defined as an elevation in TSH above the upper limit of the reference range with normal serum T4 and T3 concentration. In our study 2.4% patients had a high level of TSH(15uIU/ml). Subclinical hyperthyroidism is defined biochemically as normal serum T4, and T3 and decreased TSH concentration and 4.8% patients were classified as such. Though this condition represent the earliest stages of thyroid dysfunction treatment may be beneficial to patients with serum TSH lower than 0.1mIU/L or higher than 10mIU/L(13,14). Cytologically as expected all these patients had nodular goitres.

Approximately 80% of patients had FNA cytological diagnosis of nodular goiter and this corresponded with a hormonal profile of euthyroidism (47.6%) followed by compensated hyperthyroidism (16.7%), hyperthyroidism (11.9%), subclinical hyperthyroidism (4.8%) and subclinical hypothyroidism (2.4%) 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. 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 (15).

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 (12). In this study only one patient had a malignancy (papillary carcinoma) but had normal serum levels of total T4, T3 and TSH concentrations (euthyroid). An abnormal TSH, T3, T4 determination decreases the suspicion but does not eliminate the possibility of malignancy in a thyroid nodule (16).

One way analysis of variance (ANOVA) was done. The goal was to determine whether serum total T4, T3 and TSH concentrations vary across the six 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. 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, free T4, and free T3 would be preferable.

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