



CYTOMORPHOLOGICAL ASSESSMENT AND THYROID FUNCTION TEST ANALYSIS : A DUAL APPROACH TO DIAGNOSE THYROID LESIONS

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ABSTRACT **Introduction:** Fine needle aspiration cytology (FNAC) is now accepted as most accurate, sensitive, specific a cost-effective, minimally invasive, low-complication non operative diagnosis for most of the thyroid lesions and is highly successful in triaging patients with solitary thyroid nodule (STN) into operative and non-operative groups. **Aim and objectives:** To study cytomorphological features of palpable midline thyroid lesions, to classify thyroid cytology smears in six categories by using TBSRTC criteria, to convey brief plan to clinicians. **Methodology:** This prospective, randomized study will be carried out in the outpatient Department of PATHOLOGY, Jhalawar Medical College and SRG Hospital & SHKBM Hospital, Jhalawar. The study will comprise of Thyroid lesion all patients of either sex and age patients admitted /attending OPD in our hospital **Result:** Maximum 42.5% study subjects had colloid goiter, 7.5% subjects each had nodular goitre , lymphocytic thyroiditis respectively, 5% subjects each had subacute thyroiditis and suspected malignancy. 5.8% subjects had hashimoto thyroiditis rest other subjects had other diagnosis. Out of 7 subjects had hashimoto thyroiditis in which 2 subjects were euthyroid, 3 subjects were hypothyroid and 2 subjects were hyperthyroid. 6 subjects had subacute thyroiditis in which 3 subjects were euthyroid and rest 3 were hyperthyroid. **Conclusions:** Majority of study subjects had colloid goiter followed by nodular goiter, lymphocytic thyroiditis, hashimoto thyroiditis, subacute thyroiditis and suspected malignancy. As per Bethesda classification majority of patients belong to category II, followed by category I, category IV, V and category VI.

KEYWORDS : TSH, T3, FNAC, Hashimoto

Introduction

Serum thyroid stimulating hormone (TSH) and thyroid ultrasonography (US) are pivotal in the evaluation of thyroid nodules, as they provide important information regarding thyroid nodule functionality and the presence of features suspicious for malignancy, respectively. Fine needle aspiration (FNA) biopsy is the most accurate and reliable tool for diagnosing thyroid malignancy and selecting candidates for surgery, particularly if performed under ultrasound guidance. The cytology findings from FNA biopsies will fall into an indeterminate category in approximately 25% of the cases, in which case malignancy cannot be safely excluded. (1)

Fine needle aspiration cytology (FNAC) is now accepted as most accurate, sensitive, specific a cost-effective, minimally invasive, low-complication non operative diagnosis for most of the thyroid lesions and is highly successful in triaging patients with solitary thyroid nodule (STN) into operative and non-operative groups. The accuracy of the FNAC analysis approaches 95% in the differentiation of the benign nodules from the malignant nodules of the thyroid gland. (2) FNAC of the thyroid swellings is reported to have a sensitivity range of 65 - 98% and a specificity of 72 - 100%. (3)

The location of the target lesion, careful searching for malignant cells and repeat FNAC are the key to successful diagnosis to plan a proper surgical management in thyroid mass. Thyroid nodules that should be considered for FNAC include any firm, palpable, solitary nodule or nodule associated with worrisome clinical features. FNAC should also be performed on nodules with suspicious ultrasonographic features; dominant or atypical nodules in multinodular goiter; complex or recurrent cystic nodules; or any nodule associated with palpable or ultrasonographically abnormal cervical lymph nodes; it should be performed on any abnormal-appearing or palpable cervical lymph nodes. (4) The distinction of the benign and malignant thyroid nodules is fundamental, as malignancy necessitates surgery, while strict patient follow-up is necessary in the case of a benign mass. FNAC is considered to be the "gold standard" in the selection of patients for surgery. (5)

Any solitary or dominant thyroid nodule larger than 1 cm should have cytology done as smaller nodules carry a very low risk of morbidity. FNAC is the most cost-effective invasive pre operative investigation, whose simplicity and safety justify its use for "selective" surgery and is considered the "gold standard" in the management of thyroid nodules. (6)

The Bethesda System for Reporting Thyroid Cytopathology, published in 2007, has been widely used. It classifies the cytological results into 6 categories and presents their respective malignancy rates and approaches to treatment. These 6 categories are: nondiagnostic or unsatisfactory (ND, Bethesda I), benign (Bethesda II), atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS, Bethesda III), follicular neoplasm or suspicious for follicular neoplasm (FN/SFN, Bethesda IV), suspicious for malignancy (SM, Bethesda V), and malignant (Bethesda VI) (7)

Laboratory artifacts resulting in discrepant thyroid function tests (TFTs) may arise due to anti-T4 or heterophile antibodies or abnormal concentration or affinity of TH transport proteins (TBG, albumin, and transthyretin); these conditions are significantly more frequent than genuine IST, and as such, result in real potential for misdiagnosis. The coexistence of a primary thyroid disorder is frequent, particularly in areas of mild/moderate iodine deficiency and might cause additional diagnostic uncertainty. Finally, once a diagnosis of IST is confirmed, TSHomas must be differentiated from RTH β , due to different management and therapy of these disorders (8)

Hence this study was conducted to use both cytomorphological methods and thyroid function test in the diagnosis of thyroid lesion.

Aim and objective

To study cytomorphological features of palpable midline thyroid lesions, to classify thyroid cytology smears in six categories by using TBSRTC criteria, to convey brief plan to clinicians.

Methodology

This prospective, randomized study will be carried out in the outpatient Department of PATHOLOGY, Jhalawar Medical College and SRG Hospital & SHKBM Hospital, Jhalawar. The study will comprise of Thyroid lesion all patients of either sex and age patients admitted /attending OPD in our hospital

SAMPLE SIZE:

We calculate sample size as per the prevalence of thyroid lesion. As per the previous studies the prevalence of thyroid lesion was 15.8%, (based on study by Dalia Dauksiene et al (9)), the maximum error in the estimate we are willing to tolerate, say $\pm 7\%$, at 2-sided test with 95% confidence level ($\alpha=5\%$) and design effect =1, expected sample size is 105 patients. But to increase power we increase it upto 120 so total sample size 120 were taken

INCLUSION CRITERIA:

- All cases of midline neck thyroid swelling which are moving with deglutition.

EXCLUSION CRITERIA:

- Subjects presenting with mid line neck swelling not moving with deglutition.
- Uncooperative subjects
- Non thyroidal midline neck swellings.

METHODOLOGY-

After obtaining approval and clearance from the institutional ethical committee , only those patients meeting the inclusion and exclusion criteria were enrolled for the study. Informed consent was obtained from each participant. After enrollment the following parameters were considered and/or measured in all patient's: name age, gender, religion, occupation, address, General physical examination. Detailed clinical data, family history, personal history, physical examination and relevant investigations were noted.

Thyroid function tests:- T3, T4 and TSH. Following values were considered for thyroid status

Normal ranges:-

- T3(total) - 80-200 ng/dl
- T3(free) – 4-10 ng/dl
- T4(total) – 4.5-12.5 microgm/dl
- T4(free) – 0.8-2.8 ng/dl
- TSH – 0.5-4.70 microIU/ml

FNAC was done using 5 ml/10 ml disposable syringe with 22G/25G needle for each prick and for each patient with aseptic precaution. No local anesthetic was used.

The needle was inserted into the palpable lesions, depending on the adequate material obtained, single or multiple passes may be done. Air dried and wet fixed smears were used to stain the slides for romanowsky group of stain and papanicolaou stain respectively. Slides were immersed in a fixative 95% methyl alcohol . Slides were stained with Field and Papanicolaou stain.

Result

TABLE-1: DISTRIBUTION OF PATIENTS ACCORDING TO FNACASPIRATE

FNAC ASPIRATE	FREQUENCY	PERCENT
ACELLULAR FLUID	4	3.3
BLOOD MIXED	77	64.2
BLOOD MIXED COLLOID	2	1.7
COLLOID LIKE	4	3.3
COLLOID ASPIRATE	27	22.5
COLLOID MATERIAL	1	0.8
COLLOID MIXED ASPIRATE	1	0.8
CYST FLUID ONLY	2	1.7
JELLY LIKE FLUID	2	1.7
TOTAL	120	100

In the above table, majority of 77(64.2%) patients had blood mixed and the remaining 27(22.5%) had colloid aspirate, 4(3.3%) had in both acellular fluid and colloid like, 2(1.7%) had in each blood mixed colloid, cyst fluid only and jeely like fluid and 1(0.8%) had in each colloid material and colloid mixed aspirate.

TABLE-2 : DISTRIBUTION OF PATIENTS ACCORDING TO FNAC DIAGNOSIS

FNAC DIAGNOSIS	FREQUENCY	PERCENT
BENIGN CYSTIC LESION	1	0.8
BENGIN FOLLICULAR	2	1.7
BENGIN THYORID LESION	3	2.5
CHRONIC GRANULOMATOUS REGION	2	1.7
COLLOID CYST	5	4.2
COLLOID GOITRE	51	42.5

FOLLICULAR LESION	2	1.7
FOLLICULAR NEOPLASM	3	2.5
Hurthle neoplasm	1	0.8
HASHIMOTO THYROIDITIS	7	5.8
LYMPHOCYTIC THYROIDITIS	9	7.5
LYMPHOMA	1	0.8
MEDULLARY THYROID CARCINOMA	1	0.8
NODULAR GOITRE	9	7.5
NON-DIAGNOSTIC	8	6.7
Papillary carcinoma	1	0.8
POORLY DIFFERENTIATED CARCINOMA	1	0.8
SUBACUTE GRANULOMATOUS THYROIDITIS	1	0.8
SUBACUTE THYROIDITIS	6	5.0
SUSPECTED malignancy	6	5.0
TOTAL	120	100

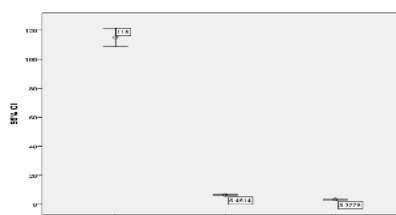
The above table, shows the FNAC diagnosis. Maximum 42.5% study subjects had colloid goiter, 7.5% subjects each had nodular goiter , lymphocytic thyroiditis respectively, 5% subjects each had subacute thyroiditis and suspected malignancy. 5.8% subjects had hashimoto thyroiditis rest other subjects had other diagnosis. Out of 7 subjects had hashimoto thyroiditis in which 2 subjects were euthyroid, 3 subjects were hypothyroid and 2 subjects were hyperthyroid. 6 subjects had subacute thyroiditis in which 3 subjects were euthyroid and rest 3 were hyperthyroid.

TABLE-3 : DISTRIBUTION OF PATIENTS ACCORDING TO BETHESEDA CATEGORY

BETHESEDA CATEGORY	FREQUENCY	PERCENT
I	8	6.7
II	94	78.3
III	4	3.3
IV	5	4.2
V	5	4.2
VI	4	3.3
TOTAL	120	100

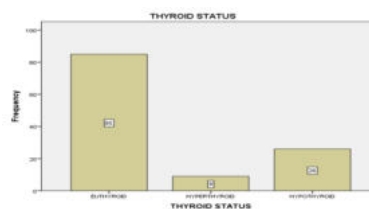
In the above table, majority of 94(78.3%) patients belong to category II and the remaining 8(6.7%) belong to category I, 5(4.2%) belong to category IV and V and 4(3.3%) belong to category VI.

GRAPH-1: DISTRIBUTION OF PATIENTS ACCORDING TO THYROID FUNCTION TESTS:



In the above table, the mean of T3 was 115 ng/dl with standard deviation 35.17, the mean of T4 was 6.46 ng/dl with standard deviation 2.78 and the mean of TSH was 3.37 ng/dl with standard deviation 1.92.

GRAPH- 2: DISTRIBUTION OF PATIENTS ACCORDING TO THYROID STATUS:



In the above table, majority of 85(70.8%) patients had Euthyroid and the remaining 26(19.2%) had hypothyroid and 26(19.2%) had hyperthyroid 9(7.5%).

TABLE- 4: COMPARISON OF PATIENTS BETWEEN BETHESDA CATEGORY AND T3:

Bethesda category	T3 ng/dl				P-VALUE
	MEAN	STD. DEV	95% CI		
			LOWER	UPPER	
I	113.62	31.78	56	155	0.7
II	116.99	35.88	44	190.0	
III	96	37.05	64	142	
IV	95.2	36.75	66	156	
V	123.2	38.69	58	162	
VI	104.5	11	88	110	

In the above table, there is an insignificant mean difference in T4 group according to Bethesda category with p-value >0.05.

TABLE 6 : COMPARISON OF PATIENTS BETWEEN BETHESDA CATEGORY AND THYROID STATUS:

Bethesda category	N	T4 ng/dl		95% CI		P-
		MEAN	STD. DEV	LOWER	UPPER	
II	94	6.65	2.91	1.00	13.80	
III	4	4.83	2.45	2.33	7.80	
IV	5	4.68	1.94	2.60	6.40	
V	5	5.92	1.56	3.30	6.90	
VI	4	5.92	0.65	5.60	6.90	

In the above table, 94 study subjects were in Bethesda category II, in which 66 study subjects were euthyroid, 9 subjects were hyperthyroid, 19 subjects were hypothyroid, out of 8 Bethesda I study subjects 6 were euthyroid, 2 were hypothyroid, out of 5 study subjects with Bethesda IV category 3 subjects were euthyroid and rest 2 subjects were hypothyroid, there is an insignificant association between thyroid status and Bethesda category with p-value >0.05.

Discussion

Although most thyroid function test patterns are easy to interpret, and concordant with clinical assessment of thyroid status, a small but important subset of patients exhibit results that are either discordant with the clinical picture or not congruent with each other. In such cases, a structured approach to further assessment is essential to avoid unnecessary/inappropriate investigation and treatment. Once confounding influences [e.g. physiological changes of age, pregnancy, intercurrent (non-thyroidal) illness or concomitant medication use] have been excluded, close liaison with the clinical biochemistry laboratory is required to systematically exclude thyroid hormone and TSH assay interference. Only then should further investigation for rare acquired and genetic causes of anomalous/discordant TFTs be considered.

In the current study majority had colloid goitre which was seen in 42.5%, followed by nodular goitre in 7.5%. In a study by C.K. SANG et al (10) majority of patients had nodular goiter of which 47.6% had euthyroidism, 16.7% had biochemical euthyroidism. In a study by M. S. Siddegowda et al (2016)(11) the non-neoplastic lesions were more common (333/350; 95.1%), with colloid goitre (142/333; 42.6%) being the commonest diagnosis followed by Hashimoto's thyroiditis (126/333; 37.8%). Among the neoplastic lesions (17/350), follicular neoplasms (13/17; 76.4%) were most common followed by papillary carcinoma. In a study by Anuja Bhargava et al (2019)(12) out of 120 patients with asymptomatic thyroid swelling, based on thyroid function test majority (70.8%) were found to be euthyroid, only 4 (3.3%) patients were found to be hyperthyroid and rest 31 (25.8%) were found to be hypothyroid. In a study by Sumathi S et al (2019)(13) out of 84 cases, 34 were reported as colloid goitre (40.5%), 31 were reported as inflammatory lesion (36.8%). Of 161 benign lesions, Benign follicular lesion (Colloid/Nodular/Adenomatoid) were commonest 108(67.0%) followed by lymphocytic thyroiditis 23 (14.2%)

The cytological analysis was accompanied with serological testing of thyroid hormones. According to the guidelines put forth by ATC, serum estimation of TSH should be part of initial assessment of thyroid swellings. If overt or subclinical hyperthyroidism is present, additional evaluation is required. A higher serum TSH level, even within the upper part of the reference range, is associated with increased risk of malignancy in a thyroid nodule, as well as more advanced stage thyroid cancer. Thyroid diseases can present with different thyroid function status, depending on the stage and extent of the disease development. While correlating the thyroid disease with hormonal status most patients with colloid goitre had euthyroid status. This finding was similar to a study done by Junu et al (14). Enlargement of the thyroid, or goiter is caused by impaired synthesis of thyroid hormone, which is most often the result of dietary iodine deficiency leading to increase in TSH levels. Increased TSH stimulates the thyroid follicular cells causing compensatory hypertrophy and hyperplasia, which finally culminates in gross enlargement of the gland. Anatomic increase in size of thyroid leads to increased hormonal production and achievement of euthyroid status in most of the individuals. In the present study the mean of T3 was 115 ng/dl with standard deviation 35.17, the mean of T4 was 6.46 ng/dl with standard deviation 2.78 and the mean of TSH was 3.37 ng/dl with standard deviation 1.92. The mean T3 level in Bethesda category II was 116.99 ng/dl, for category I it was 113.62 and for category VI it was 104.5. In a study by Daniel Asmelash et al (2019)(15) the mean concentration of T3, T4, and TSH was 110.6, 2.35 and 1.69, respectively.

In the current study majority of 85(70.8%) patients had Euthyroid and the remaining 23(19.2%) had hypothyroid and 9(7.5%) had hyperthyroid. In a study by C.K. SANG et al (2006)(10) 11.9% had hyperthyroidism, 4.8% had sub-clinical hyperthyroidism and 2.4% had sub-clinical hypothyroidism. In a study by Daniel Asmelash et al (2019)(15) of all thyroid dysfunction, subclinical hyperthyroidism and hyperthyroidism were 36.6% and 55.4%, respectively. In a study by M. S. Siddegowda et al (2016)(11) Thyroid hormone profile was done in 327 patients, of which 173 were euthyroid, 134 were hypothyroid and 20 were hyperthyroid.

In the present study majority of 94(78.3%) patients belong to category II and the remaining 8(6.7%) belong to category I, 5(4.2%) belong to category IV and V and 4(3.3%) belong to category VI. 94 study subjects were in Bethesda category II, in which 66 study subjects were euthyroid, 9 subjects were hyperthyroid, 19 subjects were hypothyroid, out of 8 Bethesda I study subjects 6 were euthyroid, 2 were hypothyroid, out of 5 study subjects with Bethesda IV category 3 subjects were euthyroid and rest 2 subjects were hypothyroid, there is an insignificant association between thyroid status and Bethesda category with p-value >0.05. In a study by Safa Alshaikh et al (2018)(16) Categories were as follows: 10.1% were Category I (nondiagnostic), 68.8% Category II (benign), 12.4% were Category III (atypia of undetermined significance), 2.9% were Category IV (suspicious for follicular neoplasm), 2.6% were Category V (suspicious for malignancy), and 4.1% were Category VI (malignant). Surgery was done on 126 nodules from 119 patients with an overall rate of malignancy of 27.8% (35/126 nodules). In a study by Busra Yaprak Bayrak et al (2020)(17) the rates of malignancy among patients who underwent surgery were 25% for category III and 27.6% for category IV, with no significant differences between categories (p=0.67).

Conclusion

On the basis of our study, we can conclude that majority of patients had midline neck swelling. Most of them had firm gross swelling and without any apparent clinical features. Those who present with clinical features Pain on swallowing, Hoarseness of voice, were common features. Majority of patients were Euthyroid followed by hypothyroid and hyperthyroid. On FNAC diagnosis majority of study subjects had colloid goiter followed by nodular goiter, lymphocytic thyroiditis, Hashimoto thyroiditis, subacute thyroiditis and suspected malignancy. As per Bethesda classification majority of patients belong to category II, followed by category I, category IV, V and category VI.

Limitation

The present study was conducted for limited time duration and USG guided FNA is recommended for very small thyroid lesions. However, all clinical data along with other investigations such as thyroid profile and thyroid scan should be assessed for definitive diagnosis.

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