Original Article

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Cytomorphological Assessment and Thyroid Function Analysis – A Dual Approach to Diagnose Thyroid Lesions



M. S. SIDDEGOWDA, JASNEET KAUR SANDHU, S. SHIVAKUMAR

ABSTRACT

Introduction: Thyroid lesions are a commonly encountered clinical problem in all age groups. Accurate preoperative assessment guides the clinical management and improves the patient outcome.

Aim: To describe the cytomorphological features of palpable thyroid nodules using fine needle aspiration cytology (FNAC) along with the assessment of thyroid hormonal status of the patient. The study also tried to assess the sensitivity, specificity and diagnostic accuracy of FNAC as an initial diagnostic modality for thyroid lesions.

Materials and Methods: The study was conducted in the Department of Pathology of a teaching hospital during July 2013 to December 2014 and included 350 patients with thyroid lesions. Cytological assessment was done using FNAC along with serological assessment of thyroid hormones and cyto-histopathological correlation.

Results: A total of 350 patients underwent cytomorphological assessment using FNAC, of which most were females with most patients being in the age group of 21 to 40 years. Non-neoplastic lesions were more common, with colloid goitre being the most common diagnosis. Among the neoplastic lesions, follicular adenoma was the most common diagnosis. Thyroid hormone profile was done in 327 patients. Same thyroid disease showed different thyroid status in different patients. Cyto-histopathological correlation was done in 28 patients. The sensitivity of FNAC in the present study was 87.5%, specificity was 91.7%, positive predictive value (PPV) was 93% and negative predictive value (NPV) was 85%. The diagnostic accuracy of FNAC in diagnosing thyroid lesions was 89.3%.

Conclusion: FNAC is a sensitive and specific technique for the diagnosis of thyroid lesions. FNAC in conjunction with hormonal analysis helps in proper patient assessment and management.

Keywords: Cyto-histopathological correlation, Hashimoto's thyroiditis, Nodular colloid goitre, TSH

INTRODUCTION

The prevalence of thyroid swelling ranges from 4% to 7% in the general adult population and from 0.2% to 1.8% in children. The clinical importance of thyroid nodule diagnosis rests with the need to exclude thyroid cancer, which occurs in 7%-15% of the cases depending on age, sex, radiation exposure, family history and miscellaneous other factors [1]. Pre-operative distinction of benign lesions is of paramount importance to avoid unnecessary surgery.

The normal thyroid gland is composed of follicles, which are the site for synthesis and storage of thyroid hormones. An intact hypothalamus-pituitary-thyroid axis and a steady source of iodide are essential for the normal thyroid hormone synthesis. The hypothalamus secretes thyrotropin-releasing hormone (TRH), which in turn stimulates the thyrotrophs of anterior pituitary to secrete thyroid-stimulating hormone (TSH). Under TSH stimulation, iodine enters the follicular cells as inorganic iodide and is transformed into thyroid hormones, thyroxin (T4) and 3,5,3'-triiodothyronine (T3) through a series of metabolic steps. In circulation, most (70%) of the T4 circulates bound to thyroid binding globulin (TBG) followed by transthyretin (20%) and albumin (10%). While most of the circulating T3 is bound to TBG, it does so with a tenfold-reduced affinity as compared with that of T4. A small percentage of T4 and T3 remain unbound and are metabolically active [1].

Thyroid diseases may be classified on the basis of clinical features of the patient and the hormone profile as hyperthyroid, hypothyroid and euthyroid [1]. However, in majority of the patients, symptoms are subtle in presentation, so the onus lies on biochemical testing and cytological evaluation to make a diagnosis [2]. The various thyroid diseases can have either of the above status at different stages of the disease development in the same patient.

In the past 5 to 6 decades, FNAC has been increasingly utilized for the investigation of thyroid lesions. FNAC by

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aspiration or non-aspiration technique is a simple outpatient procedure performed on thyroid swellings, which has the sensitivity of 93.4%, specificity of 74.9% and positive predictive value of 98.6% [3].

The use of FNAC in conjunction with thyroid hormonal profile helps in assessing the stage of the disease and deciding the treatment option for the patient. In this study, we aimed to assess the cytological findings of palpable thyroid nodules in conjunction with thyroid hormonal profile of the patient.

MATERIALS AND METHODS

A retrospective descriptive study was conducted on 350 patients in the Pathology Department of a teaching hospital after obtaining clearance from institutional ethical committee. The study included all the patients, irrespective of age and gender who presented to the cytology section with a referral for thyroid fine needle aspiration cytology during July 2013 to December 2014. The patients who presented for a repeat FNAC due to any cause were excluded from the study. Out of 350 patients, histopathological confirmation was available for 28 patients. At the time of presentation to the Department, history was obtained and clinical examination of the thyroid lesion was performed.

After consent of the patient (or the guardian/parent, in children below 18 years of age, whichever applicable), using aseptic precautions FNAC was performed by aspiration and non-aspiration technique by 23-gauge needle with 10ml syringe. On aspiration of cystic swellings, the fluid was centrifuged, following which sediment smears were prepared. These smears were fixed in 95% alcohol (wet fixation) for haematoxylin and eosin staining and air-dried for May Graunwald Geimsa staining. The results of serological estimation of T3, T4, and TSH using enzyme linked fluorescent assay (ELFA) by VIDAS Biomeriuex, which was already done on clinician's directions, were collected from the patient record at the time of FNAC.

Plan of Data Analysis: True positivity was considered when the lesion was found to be malignant on both FNAC and evaluation of post-surgical specimen. False positive were those cases wherein cytology was reported as malignant but on evaluation histopathologically the lesion turned out to be of benign nature. True negative (TN) were benign on both cytology and histopathology. False negative (FN) were negative on cytology but positive for malignancy on histopathology. Sensitivity was the detection of disease when it was actually present and was a measure of detection of thyroid cancer by FNAC in our study. Similarly specificity was defined by the ability of FNAC to exclude malignancy, that is, diagnose benign lesions. Diagnostic accuracy was calculated by using sensitivity and specificity. The statistical formulae used were as follows [4]:

- Sensitivity = TP / (TP + FN) x 100
- Specificity = TN / (TN + FP) x 100

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• Accuracy = TP + TN / Total No. of Patients x 100 Descriptive statistics were assessed using SPSS 20.

RESULTS

The study included 350 patients with palpable thyroid lesions, of which, 334 (95.4%) were females and 16 (4.6% were males) [Table/Fig-1]. The age of the patients in the study ranged from 11years to 80 years. Most of the patients belonged to the age group of 21years- 40 years.

FNAC was performed by aspiration and non-aspiration technique on all 350 patients with serological thyroid hormone estimation on 327 patients. 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 [Table/Fig-2-7].

Age group (in years)	Male	Female	Total	
0-20	05	035	040	
21-40	05	191	196	
41-60	06	087	093	
61-80	00	021	021	
Total	16	334	350	
[Table/Fig_1]: Age and gender distribution of natients with nalpable				

thyroid lesions (n=350).



[Table/Fig-2]: Hormonal status in various thyroid lesions.



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[Table/Fig-4]: Microphotograph showing polymorphous population of lymphoid cells with thyroid follicular cells (MGG, x10); Inset: Hurthle cells in a background of lymphoid cells (H & E, x40). [Table/Fig-5]: Microphotograph showing monomorphic thyroid follicular cells in sheets, clusters and microfollicular pattern with scant colloid (MGG, x10); Inset: Microfollicular pattern (MGG, x40). [Table/Fig-6]: Microphotograph showing neoplastic thyroid follicular cells arranged in papillary structures (H & E, x10); Inset: Nuclei showing powdery chromatin, nuclear groove and intranuclear inclusion (H & E, x40).

Non-neoplastic lesions	Age group (in years)			
(n=333)	1-20	21-40	41-60	61-80
Nodular goitre	22	76	36	08
Benign cyst	00	07	06	01
Lymphocytic thyroiditis	02	13	05	01
Hashimoto's thyroiditis	10	82	29	05
Adenomatoid hyperplasia	04	07	11	03
Primary Hyperplasia	01	02	02	00
Neoplastic lesions	Age group (in years)			
(n=17)	1-20	21-40	41-60	61-80
Follicular	01	09	02	01
Papillary	00	01	00	01
Anaplastic	00	00	01	01
[Table/Fig-7]: Distribution of FNAC finding of thyroid lesions (n=350)				

Thyroid hormone profile was done in 327 patients, of which 173 were euthyroid, 134 were hypothyroid and 20 were hyperthyroid. Different thyroid status was observed in the same disease process in different patients [Table/Fig-2].

In the present study, out of the 350 patients, surgical intervention was done for 28 patients. In the surgical specimens, neoplastic lesions (16/28; 57.1%) were more common than non-neoplastic lesions (12/28; 42.9%). Among the neoplastic lesions, 12 were of follicular type (10-follicular adenoma, 02- follicular carcinoma), 02 were papillary carcinoma, 01 was papillary carcinoma with goitre [Table/Fig-8,9]. and 01 was follicular adenoma with colloid goitre [Table/Fig-10]. In the neoplastic lesions, cytology correlated positively in all cases of follicular type and 02 cases of papillary carcinoma. One case of goitre with papillary carcinoma was diagnosed as goitre on cytology and another case of goitre with follicular adenoma was diagnosed as goitre with Adenomatoid hyperplasia on cytology. Cytology correlated positively in 11 cases of non-neoplastic lesions (07-multinodular goitre, 04-goitre with cystic change),



[Table/Fig-8]: Microphotograph showing papillary carcinoma with multinodular goiter (H & E, x4). **[Table/Fig-9]:** Microphotograph showing a papilla with fibrovascular core and lined by thyroid follicular cells (H & E, x10); Inset: Nuclear features of papillary carcinoma-chromatin clearing with nuclear grooves (H & E, x100).



[Table/Fig-10]: Microphotograph showing encapsulated follicular adenoma (H & E, x10).

whereas 01 case of adenomatoid hyperplasia in multinodular goitre was diagnosed as follicular neoplasm on cytology [Table/Fig-11].

Thus, the sensitivity of FNAC in the present study was 87.5%, specificity was 91.7%, positive predictive value (PPV) was 93% and negative predictive value (NPV) was 85%. The diagnostic accuracy of FNAC in diagnosing thyroid lesions was 89.3% [Table/Fig-12].

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FNAC	His	Total		
	Neoplastic	Non-neoplastic		
Neoplastic	14	01	15	
Non-neoplastic	02	11	13	
Total	16	12	28	
[Table/Fig-11]: Cytohistological correlation (n=28).				

DISCUSSION

Thyroid lesions are a common problem encountered in clinical practise. In our study, during 18 months, 350 cases of palpable thyroid nodules underwent FNAC by aspiration and non-aspiration technique and 327 of them had serological estimation of thyroid hormones. Of the 350 patients, most lesions were observed in females (95.4%), which correlate with similar studies done by Sheela et al., [5], CK Sang et al., [6], Gupta R et al., [7] and Rupam et al., [8]. The age of the patients in the study ranged from 11 years to 80 years. Most of the patients were in the age group of 21 to 40 years. Sheela et al., [5], CK Sang et al., [6] and Gupta R et al., [7] have reported similar findings.

Thyroid enlargement needs thorough investigation, mainly to rule out malignancy and thyroiditis. The recommendation of American Thyroid Association (ATC) (2015) state that for any thyroid nodule > or equal to 2cm, ultrasound should be the initial investigation followed by FNAC [9]. However, in our study, the initial investigation following clinical examination and history taking was FNAC, which is the scenario in most of the government hospitals in India. Out of the 350 patients, 333 had non-neoplastic lesions and 17 had neoplastic pathology, similar to studies done by Silverman JF et al.,[10], Uma H et al.,[11], Sengupta et al.,[12] and Gupta R et al.,[7]. Among the non-neoplastic lesions, colloid goitre was most commonly encountered followed by Hashimoto's thyroiditis. These findings are in concordance with studies done by Sheela et al., [5], CK Sang et al., [6] and Gupta R et al., [7]. Among the neoplastic lesions, follicular lesions were most common (13/17; 76.4%) followed by papillary carcinoma, similar to studies of Sheela et al., [5] and CK Sang et al., [6].

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 [9].

Thyroid diseases can present with different thyroid function status, depending on the stage and extent of the disease development. In our study of the 350 patients, serological testing was available in 327 patients and most of the patients were euthyroid (173/327). While correlating the thyroid disease with hormonal status most patients with colloid

goitre (114/142) had euthyroid status. This finding was similar to a study done by Junu et al.,[13]. 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 [14].

In patients with Hashimoto's thyroiditis, most of the patients were hypothyroid (96/126; 76.2%). Minnu Prasannan et al.,[15] have reported similar findings. Usually Hashimoto's thyroiditis presents as painless thyromegaly in middle aged woman. In most of the cases, it progresses over a period of time to subclinical and then clinically overt hypothyroidism. However, in some cases, it may be preceded by a transient hyperthyroid state (hashitoxicosis) due to destruction of follicles and subsequent rise in the free T3 and T4 along with fall in TSH and diminished radioactive iodine uptake [14]. Cytologically in our study, oxyphil cells, abundant polymorphous population of lymphocytes, scant or no colloid were observed. Some cases also showed the presence of multinucleated giant cells and epithelioid cells, similar to the findings of Gharib et al., [16]. Hence concurrent FNAC and estimation of thyroid hormonal status helps the clinician in proper management of such patients.

Among the non-tested individuals, neoplastic lesions were more than non-neoplastic lesions. It could be due to the fact that neoplastic lesions are usually present in euthyroid individuals as asymptomatic nodules.

In the present study, histopathological correlation was available in 28 cases, of which 16 were neoplastic and 12 were non-neoplastic lesions [Table/Fig-11]. In neoplastic lesions, positive cyto-histological correlation was observed in 14 cases. One case of goitre with papillary carcinoma was diagnosed on cytology as goitre [Table/Fig-3]. Stromal degenerative fragments can mimic bubble-gum type colloid and large amounts of loose colloid may also be seen in papillary carcinoma, causing confusion in the diagnosis [17]. Another case of nodular colloid goiter with co-existent follicular adenoma was diagnosed as goiter with adenomatous hyperplasia in our study. Pavithra et al., observed similar findings in their study [18]. This could be explained by the presence of colloid rich areas in the goitrous nodule and could have been avoided by aspiration from multiple sites in a setting of multinodular lesion. In difficult cases, presence of nuclear crowding and overlapping with monomorphic population of cells in predominantly microfollicular pattern and absence of colloid favours the diagnosis of malignancy [19].

Among the non-neoplastic lesions, positive correlation was observed in 11 cases and one case of Adenomatous

hyperplasia in multinodular goitre was reported as follicular neoplasmon cytology. Hyperplastic nodules are characterized by the presence of variable cellular patterns varying from honeycomb or follicular arrangement to singly scattered thyroid follicular cells. These cells display scant to abundant (oncocytic change), mildly vacuolated cytoplasm and small dark staining nuclei. Watery colloid with cytoplasmic thyroglobulin blebs may also be seen. In certain cases, the presence of features like nuclear grooves, chromatin clearing with overlapping of nuclei might create diagnostic dilemma [17].

The most commonly described pitfall in diagnosing papillary carcinoma is the presence of cyst along with papillary carcinoma. However, our study has also highlighted the presence of co-existent papillary carcinoma and nodular colloid goiter without cystic change causing difficulty in diagnosis on cytology alone. This is because of the occult papillary carcinoma, which was masked by the nodular goiter (which formed the major bulk of the swelling). These kinds of lesions require a high degree of suspicion and careful examination of nuclear features in long standing goiter cases. Chen and Rossi have also emphasized the importance of nuclear features in diagnosing papillary carcinomas as compared to the presence of characteristic architectural pattern [20].

In our study, we observed a high sensitivity and specificity of FNAC in diagnosing the thyroid lesions, similar to various other studies done previously. The diagnostic accuracy in our study was 89.3%. The accuracy of FNAC is distinctly higher in centres where both procedure and interpretation are done by trained cytopathologists. FNA cytology of thyroid has a high negative predictive value, which is useful to reassure the majority of patients presenting with thyroid enlargement and can be used to plan definitive surgery. [Table/Fig-12]. Thyroid function tests and FNAC used in conjunction in selected cases complement one another.

Study	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Piromalli et al., [21]	95	98	95	97	-
Sheela et al., [5]	90	96	-	-	94
Inamullah et al., [22]	75	96	81	95	93
Rupam et al.,[8]	82.14	86.8	65.71	94.04	83.6
Lewis et al.,[23]	-	-	-	93	-
Present study	87.5	91.7	93	85	89.3
[Table/Fig-12]: Comparison of statistical analysis among various studies					

We did a retrospective audit based analysis of data and review of slides at a medical college hospital attached to

a district hospital. Due to the rural setting, patients are not able to undergo all diagnostic tests owing to economical and social reasons. The loss to follow up (due to the same reasons or) of patients referred from surrounding areas as well as the fact that most of the lesions do not require surgical intervention, also limits the number of cases wherein histopathological correlation is available. None of our cases underwent a thyroid scan or antibody panel analysis. However, in spite of the limited resources we had a high satisfactory rate of smears and FNAC proved to be a sensitive and specific diagnostic modality. The ability of FNAC to differentiate benign from malignant lesions, with the exception of follicular neoplasm, aids in deciding the management algorithm with the biochemical tests helping in making a decision on the use of combined medical and surgical modalities in individual cases.

CONCLUSION

FNAC is a simple, safe and cost effective diagnostic modality for thyroid lesions with high specificity and accuracy, especially in developing countries like India. Skillful sampling of the lesion ensures a satisfactory cytological analysis and in conjunction with thyroid hormone profile, it helps clinicians determine the course of therapy in the management of patients with thyroid nodules.

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