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Original Article

Pathology Section

Cyto-Histological Correlation of Thyroid Lesions with Estrogen and Progesterone Receptor Status on Neoplastic Lesions

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ABSTRACT

Introduction: Thyroid nodules are common clinical findings, they are more common in women and this incidence increases with age, a history of radiation exposure and with a diet which contains goitrogenic material. Fine needle aspiration cytology (FNAC) is an established technique for the investigation of thyroid nodules. The aim of the present study was to establish a correlation between the cytological features and the histomorphology of various neoplastic and non-neoplastic lesions. Secondly, we aimed to analyze the oestrogen receptor (ER) and the progesterone receptor (PR) status in malignant thyroid tumours.

Materials and Methods: The FNAC of 448 thyroid lesions was performed, with the histological correlation being available in 122 cases, over a period of 5 years. Immunohistochemistry was performed on the neoplastic lesions by the peroxidase-anti-peroxidase technique by using a mouse monoclonal antibody clone.

Results: A cyto-histological discrepancy was noted in 8 cases. On considering histological diagnosis as the gold standard, the overall accuracy was found to be 92.9%.The male:female ratio was 1:7.2 and the maximum number of cases belonged to the nodular colloid goiter category (82.36%). An immunohistochemical analysis for ER and PR was performed on 11 cases of thyroid malignancy, with a single case of papillary carcinoma showing focal nuclear positivity for the progesterone receptors.

Conclusion: The focal nuclear PR positivity which was seen in a single case cannot predict the biological behaviour of the tumour and thus, the use of anti-oestrogenic drugs like tamoxifen is questionable for the management of thyroid cancer. This needs to be confirmed further by taking up similar studies with a larger number of cases.

Key Words: Thyroid, FNAC, Cyto-Histological Correlation, Estrogen receptor, Progesterone receptor

INTRODUCTION

Thyroid nodules are common clinical findings and they have a reported prevalence of 4% to 7%, whereas about 50% of the general population has incidental nodules on autopsy [1]. Thyroid nodules are more common in women and the incidence increases with age, a history of radiation exposure and with a diet which contains goitrogenic material. Though goitre is a common problem, cancer of the thyroid is a rare disease and the annual incidence ranges between 2-3.8 cases in females and 1.2-2.6 cases per 100 cases in males [2].

Fine needle aspiration cytology (FNAC) is an established technique for the investigation of thyroid nodules. It has an important part to play in the pre-operative diagnosis by identifying the disease process in both solitary nodules and in the diffuse enlargement of thyroid gland. Despite many advantages, FNAC has certain limitations, which include the specimen adequacy and the cytological interpretation, as the sampling is variable and not always representative. Thus, a specific diagnosis can only be arrived at after a histological examination [3].

Thyroid disorders have a predilection for the female sex. Hyper-thyroidism, hypothyroidism and thyroid neoplasms predominantly affect women. A history of one or more pregnancies, the use of lactation suppressants and oral contraceptives, increased body weight and irregular menstruation are all associated with an increased risk of thyroid cancer, thus suggesting the role of sex steroids [4]. Epidemiological studies suggest that oestrogen and progesterone may contribute to the pathogenesis of goitre, as well as thyroid tumours.

The aim of this study was to find out the pattern of the thyroid lesions which presented to our institute, with correlation between the cytological and histological features of various neoplastic and non-neoplastic thyroid lesions, wherever available. Secondly, we aimed to analyze the oestrogen receptor (ER) and progesterone receptor (PR) status in carcinomas and anaplastic tumours. A strong positivity could be of help in equivocal cases and it could be of immense use in the treatment of thyroid carcinomas by non steroidal antioestrogens like Tamoxifen.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology over a period of 5 years. FNAC of 448 thyroid lesions was performed. A histological diagnosis was available for 122 of these cases and a cyto-histological correlation was made. An immunohistochemical analysis for ER and PR was performed on 11 histopathologically confirmed cases of thyroid malignancies.

Depending on the aspirate which was obtained, a minimum of 2 smears were air dried and stained with Leishman-Giemsa stain and 2 smears were wet fixed (ethyl alcohol) and stained with the Papanicolaou and Hematoxylin and Eosin stains.

Lesions were divided into three categories which were as follows:

- Inflammatory/others
- Neoplasm and
- Inconclusive

A minimum of 2-3 smears were prepared from separate passes with the presence of at least 6-10 clusters, each with a minimum 10

follicular epithelial cells on 2 or more slides and this was considered to be adequate.

Smears which contained excess of blood, deficient or absent follicular epithelial cells, or only colloid were considered as inadequate and they were excluded from the study. A repeat FNAC was advised in such cases.

For the histological analysis, formalin fixed, paraffin embedded and H and E stained sections were studied and the cytological features were correlated with the histology sections, wherever they were available.

Suspected cases of medullary carcinoma of the thyroid were stained by Congo Red to check for amyloid and they were examined under polarized microscopy.

For immunohistochemical analysis, the sections were taken on poly-L lysine coated slides and they were incubated overnight at 37ºC. Immunohistochemistry was performed by the peroxidase antiperoxidase (PAP) technique. A mouse monoclonal antibody clone ER/PR from Biogenix was used to demonstrate the ER and PR status. The established ER/PR positive breast carcinoma tissues served as the positive controls and sections from the normal parenchyma of the thyroid were taken as negative controls [5].

The nuclear and cytoplasmic staining was recorded and the assessment of staining intensity was done by using the Leader's category score (negative, weak, moderate and strong) [6].

RESULTS

A total of 488 thyroid lesions were studied. This included 448 FNACs [Table/Fig-1], whereas 40 cases had only a histopathological diagnosis. A cyto-histological correlation was available in 122 of the 448 cases.

The age of the patients ranged from 8 years to 80 years. There was a female predominance, with our study population consisting of 87.7% (429/488 cases) females and 12.3% (59/488.cases) males. The male : female ratio was 1:7.2.

Thyroid function tests were available in 140 cases. The maximum number of cases – [93(66.4%)] were found to be euthyroid, followed by an almost equal preponderance in the hypothyroid and the hyperthyroid category. In the euthyroid category, the commonest diagnosis on FNAC was nodular colloid goitre.

A cytohistological correlation was available in 122 cases, with the FNAC findings correlating with the histological diagnosis in most of the cases. 8 of the cases showed a discrepancy [Table/Fig-2]. On considering histopathology as the gold standard for the diagnosis, the 'p' value (Chi-square test) for the cyto-histological correlation was found to be significant (P=.003) and the accuracy of the test was found to be 92.9% in diagnosing the thyroid lesions.

12 cases were placed in the category of follicular neoplasms on the basis of cytology. Among these, the histopathology report was available in 3 cases (25%). 2 cases proved to be of a benign nature, which were diagnosed as nodular goitre with a hyperplastic change and as marked nodular hyperplasia in a background of granulomatous thyroiditis respectively. A single case was diagnosed as follicular carcinoma with prominent capsular [Table/Fig-3] and vascular invasion.

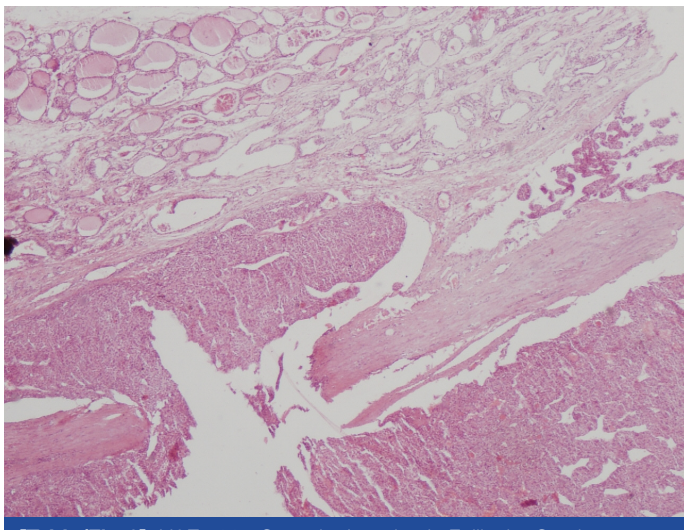
On histopathology, a total of 11 cases of malignancy were diagnosed, which included 5 cases of papillary carcinoma, 3 cases of medullary carcinoma and 3 cases of follicular carcinoma. In 5 cases of papillary carcinoma which were diagnosed by histopathology, the

Cytological Diagnosis	2006	2007	2008	2009	2010	2011	Total
Nodular Goiter	53	78	75	77	54	32	369
Thyroiditis	04	09	13	13	12	04	55
Follicular Neoplasm	02	02	03	02	01	02	12
Hurthle cell neoplasm	02	–	01	–	01	–	04
Malignant	01	–	–	02	–	–	03
Inconclusive	01	–	02	01	–	01	05
Total (%)	63 (14.6%)	89 (19.8%)	94 (20.9%)	95 (21.20%)	68 (15.17%)	39 (8.7%)	448

[Table/Fig-1]: Spectrum Of Thyroid Lesions On FNAC

S. No.	Cytological Diagnosis	Histopathology Diagnosis
1.	Follicular neoplasm	Colloid goiter with hyperplastic change
2.	Nodular colloid goiter with Hurthle cell change	Chronic thyroiditis with regenerative changes
3.	Nodular colloid goiter with hemorrhage	Chronic thyroiditis with extensive fibrosis and focal Hurthle cell change
4.	Chronic thyroiditis	Nodular colloid goiter
5.	Nodular colloid goiter with cystic change	Papillary carcinoma in background of nodular colloid goiter
6.	Chronic thyroiditis	Nodular colloid goiter
7.	Focal hyperplastic change in colloid goiter	Micropapillary carcinoma with nodular colloid goiter
8.	Follicular neoplasm	Marked nodular hyperplasia in background of granulomatous thyroiditis

[Table/Fig-2]: Cyto-Histological Discrepancy



[Table/Fig-3]: H&E,100x-Capsular Invasion in Follicular Carcinoma

cytological findings were available in 2 cases . Both the cases were missed during cytology and were diagnosed as nodular colloid goitre with hyperplastic change and colloid goitre with a cystic change. Thyroiditis was observed in the surrounding parenchyma in 4 out of 5 cases of papillary carcinoma [Table/Fig-4]. Psammoma bodies were observed in 3 out of 5 cases, which included 1 case of a follicular variant of papillary carcinoma.

All 3 cases of medullary carcinoma of the thyroid showed the presence of amyloid, which were positive for Congo red and they showed an apple green birefringence on polarizing microscopy. [Table/Fig-5].

Some of the noteworthy cases which were encountered during the study period were:

In an FNAC aspirate of the thyroid from a 35-year old female, gametocytes of Plasmodium Falciparum were seen, with associated hyperplastic changes in colloid goitre. Another case showed a follicular variant of papillary carcinoma with osseous metaplasia on histopathology [Table/Fig-6]

Immunohistochemistry was performed on 11 cases of the thyroid malignancies. Stained slides were analyzed for the following features:

1. Intensity of the staining, which was graded by using the "Leaders Category score" [6], into negative, weak, moderate or strong. A moderate and strong staining could be seen under the low power (10X) objective. Weak staining was focal and it was evident only at a higher magnification.
2. Nuclear or cytoplasmic staining:

   Out of the 5 cases of papillary carcinomas ,1 case showed a focal (weak) nuclear positivity for PR [Table/Fig-7]. 2 cases of papillary carcinomas, which included FVPC, showed cytosolic positivity for both ER and PR . The rest of the cases did not show any positivity.

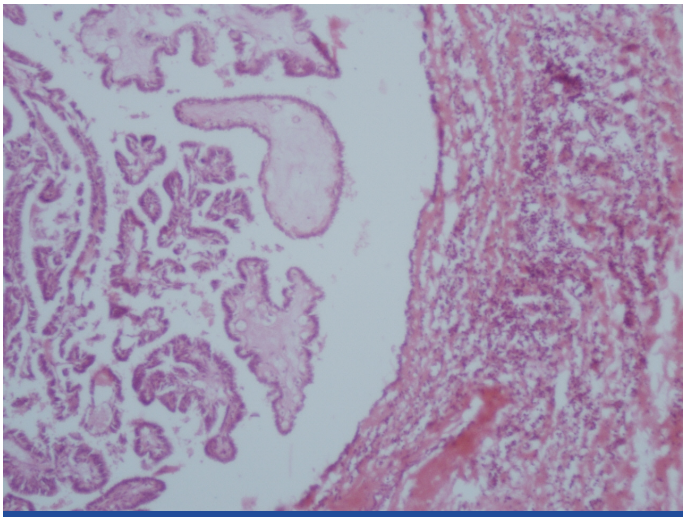
   In all the sections, a large amount of nonspecific staining was seen in the red blood cells and colloid for both ER and PR. A cytoplasmic positivity was seen in few cases and this could have been artifactual due to absence of blockade of the non specific binding by the power block.

DISCUSSION

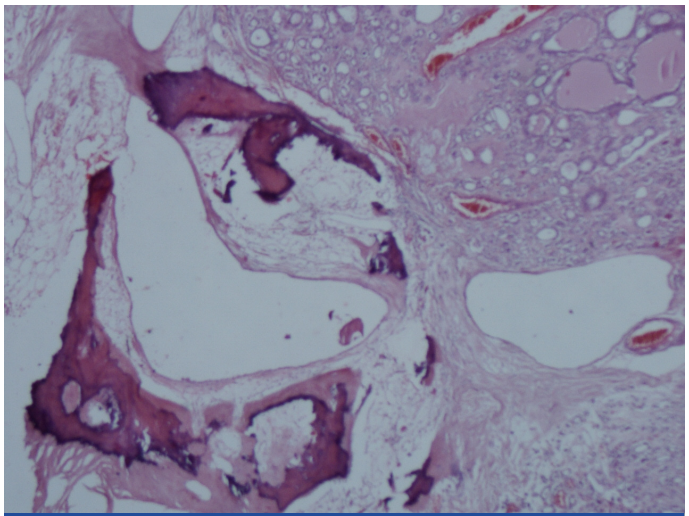
Thyroid disorders are common in India and the most common ones are goitrous enlargement and nodularity of the thyroid. FNAC and histopathology, along with clinical findings, is used to reliably distinguish benign from the malignant thyroid nodules. In our study, an overall female predominance was observed, with our study population consisting of 87.7% females and 12.3% males. This was in accordance with that which was seen in the study of Ghulam Rasool Memon et al., [7] where the thyroid disorders were found to occur more commonly in females (2.5 times) of the reproductive age group, which could have been due to the effect of oral contraceptive pills, pregnancy, lactation and other hormonal effects.

In our study, FNAC had 92.9% accuracy, 95.4% sensitivity and a specificity of 93.9% in diagnosing various disorders of the thyroid. [Table/Fig 8] illustrates and compares the results of the diagnostic accuracy of various studies with respect to the role of FNAC in the management of thyroid nodules [8,9,10,11,12].

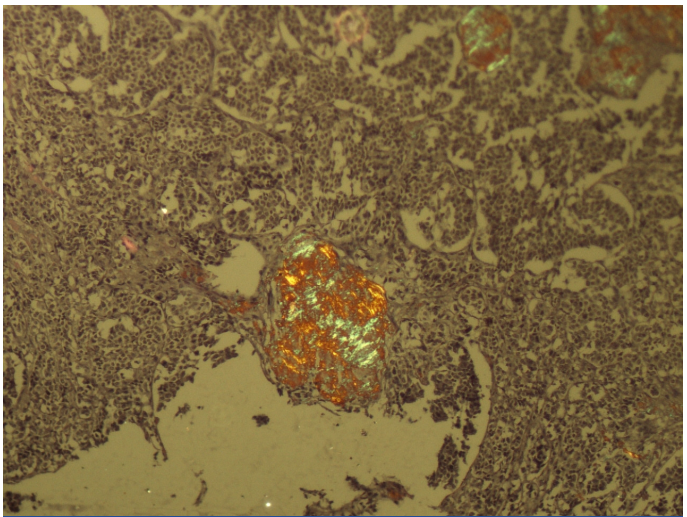
[Table/Fig-9] compares the FNAC results of the thyroid lesions in the present study with those of other studies [12,13]. In contrast to



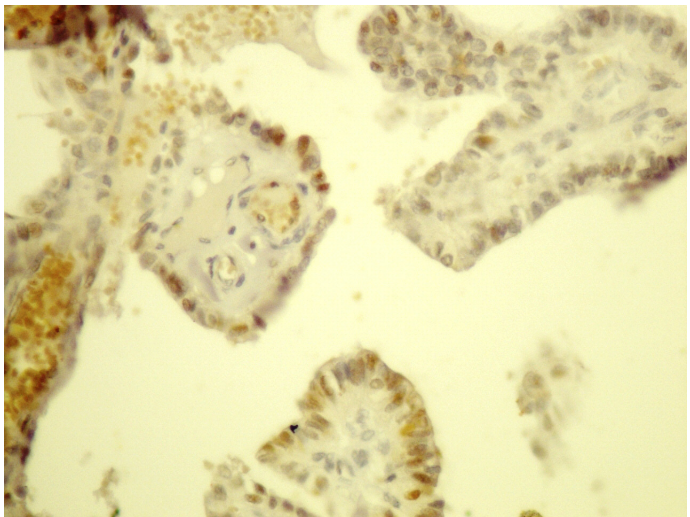
[Table/Fig-4]: H&E, 100x -Papillary Carcinoma (Left) With Chronic Thyroiditis (Right)



[Table/Fig-6]: H&E, 40x -Osseous Metaplasia in Follicular variant of papillary carcinoma



[Table/Fig-5]: Congo Red stain, 400x-Amyloid showing apple green birefringence on polarizing microscopy



[Table/Fig-7]: Focal PR Positivity In Papillary Carcinoma (X 400)

the comparative studies in [Table/Fig-9], Haggi M [14] observed an equal proportion of benign and malignant disorders, with only 31% cases being benign, 37% being malignant and 32% cases being reported as follicular neoplasms.

In the present study, a histopathological correlation was present in 122 cases of these, 8 cases showed a discordance between cytology and histopathology.

The pitfalls on FNAC which were encountered in this study were mainly with cystic lesions. Two cases of papillary carcinomas, diagnosed by histopathology, were missed on cytological evaluation, as the aspirate showed an extensive cystic change and the absence of the characteristic nuclear features. Cystic changes are known to occur in papillary carcinomas of the thyroid. Two studies which were done by Rosen IB et al., [15] and Sarda AK et al., [16] have shown that the sensitivity of FNAC in cystic neoplasms may be as low as 40% and that all the cystic lesions should be managed cautiously. Other cases in which a discrepancy was noted were follicular neoplasms as were diagnosed by FNAC vs colloid goitre with a hyperplastic nodule and thyroiditis. Settakorn [9], in 2001, reported similar discrepant findings in a few cases of adenomatous goitre vs follicular neoplasm and papillary carcinoma. The main reason behind this was that the follicular patterned lesions of the thyroid could present with a varied morphology as a hyperplastic nodule, a follicular adenoma or carcinoma and a follicular variant of papillary carcinoma which contained an admixture of the microfollicular, normofollicular and the macrofollicular patterns [17].Thyroid nodules which are diagnosed as follicular neoplasms on FNAC pose a diagnostic dilemma and they have been put into the grey zone category [18]. A study which was done by Kung IT [19] suggested certain distinguishing features on cytology which could help in differentiating the hyperplastic nodules from the follicular neoplasms. These included the cells which showed larger nuclei with prominent nucleoli and overlapping nuclei as the features of follicular neoplasms and which showed hyalinized stroma as a feature of colloid nodules.

Reference	No of pts.	Accuracy (%)	Sensitivity (%)	Specificity (%)
Present study (2006-2011)	122	92.9	95.4	93.9
Tabaqchali et al (2000) [8]	239	43.1	86.8	67
Settakorn et al (2001) [9]	1761	90	85.7	92.5
Silverman et al (1986) [10]	301	94.0	93.0	95.1
Altavilla et al (1987) [11]	257	95.1	71.4	100
Gharib & Goellner (1993) [12]	1750	98.4	98.0	99.0

[Table/Fig-8]: A comparison of FNAC results in different studies

Study	Benign/ Inflammatory	Malignant	Neoplasm	Inconclusive
Present study (2011)	94.5%	0.86%	3.34%	0.9%
Altavilla (1990) [11]	77.65%	1.31%	4.89%	16.11%
Gharib H (1993) [12]	69%	4%	10%	17%
Diana S (2008) [13]	51%	13%	27%	9%

[Table/Fig-9]: Spectrum of Thyroid Lesions on FNAC in Various Studies

Study	Year	No. of Cases	Assay Method	Er+ Cases	Pr+ Cases
Present study	2011	11	IHA	0	1
Jaklic BR et al [29]	1995	11	IHA	0	0
G.Rasool et al [7]	2005	50	IHA	0	0

[Table/Fig-10]:-Comparison of Various Studies for ER/PR Receptors in Thyroid Lessons

Papillary carcinoma was found to be the most common malignancy in our study spectrum. A zonal study which was done in Greece [20] showed an increase in the detection rates for papillary carcinoma in a given time period. A study which was done by Leung CS et al [21] on the variants of papillary carcinoma, showed that colloid was present in all the variants, but that it was seen most frequently in the follicular variant, which was statistically significant. Both thick and thin colloid were present in all the cases of papillary carcinomas in this study. Psammoma bodies were found in 3 out of 5 cases of papillary carcinomas. A study which was done by Hunt et al [22] included 29 patients who had psammoma bodies on thyroid FNAC and all these patients had a focus of papillary carcinoma on histopathology subsequently. The papillary carcinomas shared a strong association with Hashimoto's [23] and lymphocytic thyroiditis, which may be due to the RET/PTC gene rearrangement [24]. In the present study, 4 out of 5 cases of papillary carcinoma showed coexisting chronic lymphocytic thyroiditis on histopathology.

All the 3 cases of medullary carcinoma showed a positive staining for amyloid with Congo red and an apple green birefringence on polarizing microscopy. Uribe M [25] reported a positive histochemical staining in 2/2 cases in their study. A case report on the fine needle aspirates of 4 cases of amyloid goitre showed an abundant violet to pink amorphous material with the staining characteristics of amyloid in all the cases. This material was morphologically distinct from colloid. Hence, pathologists should pay close attention to the morphology of the cells which accompany amyloid, which will allow the exclusion of medullary carcinoma of the thyroid [26].

In this study, mild PR nuclear positivity was seen in one case of papillary carcinoma and two cases of papillary carcinoma showed an artifactual cytoplasmic positivity for both ER and PR .The normal thyroid parenchyma did not show any positivity for either marker and hence, it served as a negative control. A study which was done by Lewy Trenda [27] demonstrated the presence of ER on normal, benign and malignant tissues of the thyroid, with the maximum incidence of ER positivity being seen in papillary carcinoma of thyroid. No immunostaining was seen in the cases of nodular colloid goitre.

Chaudhary et al studied the presence of ER/PR in normal and neoplastic thyroid tissues by the protamine sulphate precipitation technique and they showed that carcinomas and adenomas had higher receptor content than goitre [28].

[Table/Fig-10] has compared the ER/PR positive cases of other studies which were studied by using the same immunohistochemical assay method [7,28].

Multiple factors could affect the interpretation of ER and PR immunoreactivity in the histological sections:

1. An artifactual cytoplasmic positivity may be due to the absence of a blockade of the non specific binding by the power block.
2. Thyroid sections show haemorrhage and colloid. So, despite treatment with hydrogen peroxide, a large number of red blood cells show non-specific background staining.

CONCLUSIONS

In this study, thyroid lesions were found to be predominant in females and nodular colloid goitres comprised the largest group of thyroid disorders which were diagnosed on FNAC. Papillary carcinoma was the most common thyroid malignancy which was seen. Focal nuclear PR positivity which was seen in a single case of papillary carcinoma, could not predict the biological behaviour of the tumour and thus, the use of antioestrogenic drugs like tamoxifen is questionable in the management of thyroid cancer. This needs to be further confirmed by doing similar studies on a larger number of cases.

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