Original Article

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Autoantibodies and Immune Expression of HBME 1 and Galectin 3 in Thyroid Nodules

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Abstract

Thyroid diseases are among the commonest endocrine disorders worldwide. India too, is no exception. According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases. Since mortality and morbidity in thyroid cancer is often measured over decades, there is a paucity of prospective clinical studies that are capable of evaluating various tests and to find out the cost and time saving methods which may significantly reduce patient morbidity and unnecessary surgery in benign thyroid disease. Objectives of present study was to study anti thyroperoxidase (AntiTPO) antibody, anti-thyroglobulin (anti TG) antibody and immune expression of HBME1 and galacten 3 in various thyroid lesions. This is a prospective 2 year study from January 2015 to December 2016. All cases referred to cytology section were included and individual on hormone therapy or antithyroid drugs were excluded from study. FNAC was done in all cases. Thyroid function test, anti TPO and anti TG antibodies were measured. Histopathology correlation of cases was done of resected thyroid tissue and immune markers HBME 1 and galectin 3 was done where ever required. 165 cases had cytological diagnosis reframed under Bethesda reporting and serum biomarkers level obtained. 71 cases had histopathological correlation and observed nodular goiter commonest non neoplastic lesion followed by lymphocytic thyroiditis and papillary carcinoma. HBME 1 is specific to differentiate benign and malignant lesions and galectin 3 is highly specific for papillary carcinoma. Auto-antibodies are markedly raised in autoimmune thyroiditis and papillary carcinoma. To conclude autoantibodies level estimation helps in clinical diagnosis and management of thyroid lesions. Immunohistochemistry plays vital role in confirmation of malignant lesions.

Keywords: Anti thyroperoxidase/ anti thyroglobulin/HBME1/galectin 3.

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Introduction

Thyroid diseases are among the commonest endocrine disorders worldwide. India too, is no exception. According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases [1].

Thyroid nodules are a very frequent finding, and their prevalence steadily increases with age. Nodular thyroid disease refers to the presence of a solitary nodule or multiple nodules, solid to one or more cystic lesions. It is estimated that 5%–7% of adults have clinically detectable nodules in

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the thyroid, and with the emergence of modern ultrasonographic (US) techniques detecting thyroid nodules of a few millimeters, the frequency of nodularity is estimated to be 16%–67% in unselected subjects. Most of the discovered nodules are benign; however, there are increasing incidence of cancers (2.4-fold increase), and this trend appears to be continuing. Recent population studies have shown that about 12% of adults have a palpable goitre. Autoimmune thyroid disease is probably commoner than iodine deficiency as a cause of goiter in areas that are now iodine sufficient [2,3].

Thyroid cancer is the most common endocrine malignancy and is the sixth most common cancer in women and the second most common cancer in women under 40 years of age [4].

Since mortality and morbidity in thyroid cancer is often measured over decades, there is a paucity of prospective clinical studies that are capable of evaluating various tests which may significantly reduce patient morbidity and unnecessary surgery in benign thyroid disease. The present study was taken to study the significance of FT3, FT4, TSH, anti TPO antibody, anti thyroglobulin antibody in various thyroid lesions. Cytological diagnosis of thyroid nodules, histopathological correlation and to study immune marker expression of HBME-1 and galectin 3 in the resected thyroid specimen in applicable cases.

Materials and Methods

This was a prospective 2 year study from January 2015 to December 2016. All patients with thyroid swelling from ENT & Surgery clinic being referred to Department of Pathology were included in the study with informed consent. Patients with thyroid swelling already on thyroid hormone therapy or antithyroid drugs were excluded from the study. Informed consent was obtained and then detailed clinical history was noted. Thorough clinical examination was carried out. 5 ml of venous blood sample was collected in plane vaccutainer and serum markers estimation which included thyroxine (T4), tri-iodothyronine (T3), thyroid stimulating hormone (TSH), anti Thyroperoxidase (Anti TPO), Anti thyroglobulin antibody (Anti TG). Collected sample was run through Vidas biochemical analyser. Fine needle aspiration cytology (FNAC) was done and cytological diagnosis was given following The Bethesda Reporting System for Thyroid Cytology (TBRSTC). Resected thyroid tissue was grossed and processed FFPE as per standard protocol and stained with routine Hematoxylin and Eosin (H&E) stain and histopathological diagnosis given and correlation between cytological diagnosis and histopathological diagnosis was also done in available cases. Immunohistochemistry for HBME1 and galectin 3 markers was carried out in required cases as per standard protocol.

The statistical evaluation of the data was carried out using the Statistical Package for Social Sciences (SPSS® version 17.0) and Microsoft® Excel for Mac 2011 programs. In the present study, descriptive statistics as well as 95% confidence interval for a single proportion, mean, P value and sensitivity and specificity of immune markers was calculated.

Results

Total samples included in the study were 165 cases, all cases serum biomarkers ie thyroid hormones and autoantibodies were measured and noted down. In present study females outnumbered males in thyroid disease with 81% and 19% male affected. Youngest patient was 12 yrs old (Table 1).

Serum biomarkers of thyroid function that is free T3, free T4 and thyroid stimulating hormone (TSH) were with in normal range. Anti thyroperoxidase (Anti TPO) and anti-thyroglobulin (Anti-TG) levels were raised in autoimmune thyroiditis and in few cases of papillary carcinoma (Table 2). P value was calculated and observed that P value was statistically significant for anti TPO and anti Tg levels in relation with thyroiditis and papillary carcinoma (Table 3).

The Bethesda Thyroid reporting was followed for cytological reporting and observed 83 cases (51%) were benign cases Bethesda category II, 9% cases were frank malignant remaining Bethesda category VI, 9.7% cases were follicular neoplasm category IV and 30.3% cases were falling in grey area Bethesda category V and category III. Histopathological diagnosis was correlated in available cases (Table 4). Seventy one cases had histopathological correlation. Commonest histopathological diagnosis was colloid goitre (21 cases), followed by 16 cases thyroiditis, 10 cases of follicular adenoma and 24 cases were thyroid malignancy.

Papillary carcinoma was commonest malignancy with 15 cases, 4 cases of follicular carcinoma, 2 cases of medullary carcinoma and 2 cases of metastatic carcinoma both were squamous cell carcinoma deposits. One rare case of mucoepidermoid carcinoma was diagnosed.

Forty eight (48) resected thyroid specimens with morphological similar features between

benign and malignancy, those cases were subjected immunohistochemistry. HBME 1 and Galectin 3 immune expression was studied. Gal-3 stained the majority of malignant cases (89%) in comparison to benign neoplasms the difference was statistically significant (p-value <0.0001). Gal-3 expression in thyroid papillary neoplasms was found to have a sensitivity of 88.2%, specificity of 89.12%, and positive predictive value of 91.22% and negative predictive value of 78.12%. HBME is more specific

Table 1: Age distribution

Sex/age	10-20	21-30	31-40	41-50	51-60	61-70	>70	Total
Female	15	45	33	15	18	6	2	134
Male	3	6	11	6	2	3	-	31

Table 2: Thyroid Hormones and Antibodies Level in Various Thyroid Diseases

Thyroid lesion	TSH 0.4-4.0 muIU/ml	FT3 3.5-7.8 pmol/L	FT4 9 – 25 pmol/L	Anti TG <20 IU/ml	Anti TPO < 35 IU/ml
Goitre (colloid/nodular/toxic)	1.62+/-0.76	3.02+/-042	1.22+/-0.24	16.63+/-3.42	35.2+/-3.23
Lymphocytic thyroiditis	20.66+/-4.05	1.42+/-0.37	.53+/-0.16	43.25+/-7.46	63.26+/-5.96
Granulomatous thyroiditis	2.22+/-0.43	6.24+/-1.56	19.45+/-6.5	26+/-3.50	35.2+/-2.50
Graves disease	0.02+/-0.01	13.3+/-3.69	30+/-4.79	34.85+/-6.76	42.28+/-5.92
Follicular neopalsm					
Follicular adenoma	0.83+/-0.16	4+/-1.73	11.3+/-1.67	12.6+/-3.77	25+/-0
Follicular carcinoma	2+/-0	3.9+/-0.1	20+/-0	12+/-1.41	17.5+/-2.42
Papillary carcinoma	2.1 +/- 0.54	4.65+/-1.16	13.8+/-4.16	30.5+/-14.53	33.3+/-17.93
Medullary carcinoma	0.9	4	13	20	39

Table 3: p value of the serum Biomarkers

Thyroid lesion	TSH	FT3	FT4	Anti TG	Anti TPO
Goitre (colloid/nodular/toxic)	0.182	0.218	0.321	0.61	0.12
Lymphocytic thyroiditis	0.245	0.03	0.001	0.05	0.002
Granulomatous thyroiditis	0.215	0.04	0.02	0.06	0.001
Graves disease	0.05	0.04	0.003	0.015	0.025
Follicular neoplasm Follicular adenoma Follicular carcinoma	0.224	0.23	0.231	0.215	0.071
Papillary carcinoma	0.251	0.25	0.32	0.02	0.04
Medullary carcinoma	-	-	-	-	-

Table 4: Bethesda cytological reporting and histopathological correlation

Bethesda system	Includes	Cytology cases	HPR correlation Cases	Final diagnosis
I	Non diagnostic Non satisfactory	Acellular/ only fluid/ blood	24	-
II	Benign	83	24	Goitre (colloid/nodular/ toxic) Lymphocytic thyroiditis Granulomatous disease Graves disease Adenomatoid nodule
III	Atypia of unknown significance (AUS)	32	13	
IV	Follicular neoplasm/ suspicion for follicular neoplasm	16	14	Follicular neopalsm; Follicular adenoma Follicular carcinoma
V	Suspicious for other malignancy	09	20	Papillary carcinoma Medullary carcinoma
VI	Malignancy	15		Metastatic carcinoma Mucoepidermoid carcinoma
Total	-	165	71	-

Forty cases had immunohistochemistry analysis with marker HBME 1 and Galectin 3.

Table 5: IHC HBME 1 & Galectin 3

HPR diagnosis	No of cases	HMBE 1	Galectin 3
Papillary carcinoma	15	Positive	Positive (15 case)
MNG with papillary like features	12	Weak positive	Positive (1case)
Lymphocytic thyroiditis	2	Negative	Negative
Graves disease	1	Negative	Positive
Follicular carcinoma	3	Weak positive	Negative

for thyroid malignancies compared to benign neoplasms with sensitivity of 91% and specificity of 95% (Table 5).

Discussion

In present study we had 165 thyroid lesion cases in which we observed female preponderance 79%, similar to study done by K Fariba et al. [5] with 66.8% females, WeiminXu et al. [6] and Howrah et al.

Cahoon KE et al. [7] had population based cohort study which measured serum Tg, urinary iodine, TSH, anti-thyroglobulin, anti- thyroid peroxidise levels and Ultrasound to assess the presence of nodules and estimate thyroid volume and concluded that serum Tg is significantly related to presence of thyroid abnormalities as well as indicators of thyroid function and iodine deficiency and, therefore, could be used to characterize the iodine status and thyroid function of individuals in the context of epidemiological study.

In spite of iodine sufficient belt the incidence of thyroid diseases are increasing, with the literature supporting with the autoimmune cause. Antibodies levels were raised in autoimmune thyroid disease and in known fact but levels were also high in papillary carcinoma and medullary carcinoma suggesting could play role in etiopathogenesis? Similar findings was also observed by Young Ah Cho et al. [8] in "Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: a nested case–control study" Eun Sook Kim et al. [9], reported that TGAb was associated with an increased risk of thyroid cancer in thyroid nodules. Similarly, other studies also showed an analogous association with malignancy by considering positive thyroid autoantibodies as a whole, including TPOAb and TGAb. Present study also comments on auto-antibodies levels high in autoimmune thyroiditis and few cases of papillary carcinoma.

In present study, cytological diagnosis of all cases were categorised according to Bethesda reporting system thus signifying its importance for surgical management of thyroid nodules. Study done by Zarif, et al. [10]. demonstrates higher risks of malignancy in diagnostic categories (DC) I, DC II, DC III and DC IV than that of the original BSRTC definition, along with a higher specificity and positive predictive value for cancer diagnosis, and a lower sensitivity and negative predictive value.

Several immunohistochemical markers representing different components of the cell, such as the membrane, the cytoplasm, or the nucleus, have been studied in thyroid neoplasms. Some of the antibodies that have been examined include galectin-3, Hector Battifora mesothelial cell antibody (HBME-1), cytokeratin-19, RET, TTF-1, hTERT, telomerase, p27 and p53 to name a few. Two markers that have been extensively studied are galectin-3 and HMBE-1 [11].

Saleh HA et al. [12] studied immunohistochemical markers like galectin-3, HBME-1, CK19 and Ret oncoprotein to differentiate benign and thyroid nodules and concluded that immunomarkers are significantly more expressed in malignant tumours compared to benign lesions and may be of additional diagnostic value when combined with routine histology [13].

Study done by Arcolia et al. [14]: diagnostic performances of individual or combined thyroid markers demonstrated that gal1 is a useful immunohistochemical marker to discriminate malignant tumours from benign thyroid nodules. They further validate that gal3 is a sensitive marker for the diagnosis of thyroid malignancy, and add support for its combination with CK 19 and HBME 1 with the highest performance for the diagnosis of well differentiated thyroid cancer. Such combination of markers should be validated in a larger series of tissues including various subtypes of thyroid lesions [14].

Zhang et al. [15] observed in his study the potential of triple immunochemical staining to be used as an ancillary test to clarify cytologic diagnoses of indeterminate thyroid nodules.

Also demonstrated the diagnostic value of dual positive/colocalization of Galectin-3 and HBME-1 for thyroid malignancy [15]. Similarly present study highlights the importance of galectin 3 and HBME-1 immune markers in diagnosing thyroid malignancies.

Conclusion

Cumulative approach including thyroid serum markers and tissue immune marker study gives a complete diagnostic approach of thyroid lesions and Gal-3 is a promising marker in the diagnosis of PTC and its variants and HBME-1 differential expressions in thyroid carcinoma compared with benign neoplasms may also represent a promising target for therapy of thyroid cancers.

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