



ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2018; 1(1): 08-12
Received: 10-11-2017
Accepted: 13-12-2017

Vijay Kumar Singh
B.R.D Medical College,
Gorakhpur, Uttar Pradesh,
India

The utility of CK 19 & TPO expression in the diagnosis of thyroid nodules

Vijay Kumar Singh

Abstract

Introduction: Thyroid lesion can be diagnosed using histopathological criteria which allows us to differentiate between benign and malignant lesion but in some cases suspicious areas were noted in few foci. In such cases immunohistochemistry (IHC) is used to differentiate between benign and malignant lesion. Panel of two marker, CK-19 and TPO increases the diagnostic accuracy in the thyroid lesion than a single marker.

Aim: To analyze the IHC expression of TPO and CK 19 in both benign and malignant thyroid nodular lesions.

Material and Methods: Prospective observational study of 100 thyroid specimens was studied for detailed histopathological examination and expression of immunohistochemical markers thyroid peroxidase and cytokeratin-19.

Results: TPO IHC marker was expressed by all benign and non-neoplastic lesion of thyroid and absent in case of malignant lesions. While CK-19 was expressed in all malignant thyroid lesion and shows focal and weak staining in noted in benign lesions.

Conclusion: A combination of positive and negative markers is essential in distinguishing benign and malignant thyroid lesion. TPO expression indicates benign condition and loss of its expression implicates malignant transformation and strong membranous ck-19 indicates malignancy.

Keywords: TPO expression, CK-19 expression

Introduction

Palpable thyroid nodule is a discrete swelling within an otherwise apparently normal thyroid gland ^[1]. It may be found in 4-7 % of the general population and this prevalence may approach 60% when high resolution ultrasonography (USG) is used ^[2]. In India the prevalence of palpable thyroid nodule in the community is about 12.2% according to recent study ^[3]. However thyroid cancer is quite rare, and the incidence is 8.7 per 1,000,00 people per year, though this seems to be increasing over the years ^[4]. Thyroid cancer is more common in women than in man by a ratio of above 3 to 1 and has been Sixth most common cancer in women ^[5]. We aimed to assess the role of CK19 and TPO in distinguishing between benign and malignant thyroid lesions. Thyroid peroxidase (TPO), a thyroid-specific enzyme is essential for the biosynthesis of thyroid hormone. In the last few years, the diagnostic value of TPO immunodetection in thyroid neoplasms has been widely discussed, because several authors have demonstrated that TPO protein is expressed in benign tumors and normal tissue ^[6-8] but is absent or poorly expressed in a variety of thyroid follicular carcinomas ^[9-14]. Cytokeratin -19 is low molecular weight cytokeratin found in a variety of simple or glandular epithelia, both normal and their neoplastic counter parts. In the thyroid gland, normal follicular epithelium usually has shown no detectable CK19 expression ^[15-17]. It is expressed on neoplastic epithelium. The reported sensitivity and specificity using CK19 as single marker is as high as 92% and 97% respectively ^[18].

Panel of two markers, CK-19 and TPO increases the diagnostic accuracy in thyroid lesions than a single marker ^[19].

Aim: To analyze the IHC expression of TPO and CK19 in both benign and malignant thyroid nodular lesions. To Establish the diagnostic accuracy of CK-19 and TPO, as well as their association, in differentiating benign and malignant thyroid nodular lesions. To determine the overall sensitivity and specificity of TPO and CK19 in differentiating thyroid nodular lesions.

Correspondence
Vijay Kumar Singh
B.R.D Medical College,
Gorakhpur, Uttar Pradesh,
India

Material and Methods

Study design: Cross sectional study.

Selection of cases: The present study was conducted on patients coming to outpatient department and admitted in SURGERY wards of Nehru Chikitsalaya, BRD medical college, Gorakhpur. After taking informed and written consent, histopathological examination and immunohistochemical expression of TPO AND CK 19 of the thyroidectomy specimen received in the department, were analyzed. The tissue specimens preserved in the department of pathology, BRD medical college, Gorakhpur were also utilized in the study.

Duration of study: 1 Year (JULY 2016 TO JUNE 2017).

Inclusion Criteria: Lobectomy, hemithyroidectomy, subtotal and total thyroidectomy specimens.

Exclusion Criteria: Autolysed sample & Inadequate sample. Thyroidectomy specimen were fixed in 10% formal saline and subjected to histopathological examination using paraffin embedding technique. All the paraffin blocks were preserved for section cutting. Thin sections of 4-5 micron were cut after dewaxing and then stained by hematoxylin and eosin stain. Histopathological diagnosis was made and then freshly cut sections were also used for immunostaining. Present study was carried out on 100 cases of thyroid lesions to evaluate the role of TPO AND CK 19 in various lesions of THYROID.

Table 1: TPO and CK 19 scoring criteria

Features		score
Percentage of stained cells	No cells stained	0
	10-30%	1
	>30-60%	2
	>60-90%	3
	100%	4
Intensity of staining	Negative	0
	Mild	1
	Moderate	2
	Strong	3

Using these two indicators of the score, the final immunohistochemical score for extent and staining intensity were as follows:

- 0 - negative (-)
- 1-3 - weakly positive (+)
- 4-5 - moderately positive (++)
- 6-7 - strongly positive (+++)

Results were tabulated.

Statistical analysis: Appropriate statistical tools were adopted for the data analysis. Analysis was done by data sorting method, classified by tabulation and presentation by pie charts, and histograms. Statistical method such as Chi square test were employed to find out the significance of the study.

Results: Out of total 100 cases studied, 74% cases were multinodular goitre, 5% cases were of follicular adenoma, 6% cases of hashimoto's thyroiditis, 13% cases of papillary

carcinoma, while medullary carcinoma were seen in 2% cases. Total number of benign lesion were 85% and malignant lesion were 15%. Most common age group affected was between 30-49 years. It was observed that maximum number of cases were in age group of 41-50 year (42.00%) followed by the age group of 31-40 years (41.00%). Maximum number of cases of multinodular goiter (43.24%) and papillary carcinoma (46.15%) were observed in 31-40 years of age group where as the peak incidence of Follicular adenoma (60.00%) and hashimoto's thyroiditis (83.33%) were observed in 41-50 years age group and maximum cases of medullary carcinoma were found in 51-60 years age group. Out of 100 cases, 89 cases (89.00%) were female, and 11 cases (11.00%) were male. Percentage of female in multinodular goiter were present is 89%, 100% in follicular adenoma, 83% in hashimoto's thyroiditis, 84% in papillary carcinoma and 100% in medullary carcinoma. We observed that all the 85 benign thyroid lesion were positive for TPO marker while all the 15 malignant cases were negative. Among 74 cases of multinodular goitre, grade 3 staining was seen in 72 cases while grade 2 and grade 1 staining was seen in 1 case each. Among 6 cases of hashimoto's thyroiditis grade 3 of staining was seen in 4 cases and while grade 2 and grade 1 staining was seen in 1 case each. Among 5 cases of follicular adenoma 3 cases showed grade 2 staining while 2 cases showed grade 1 staining. The intensity of staining of TPO marker was strong in 72 cases of multinodular goitre while rest 2 had moderate intensity. Also 4 cases of hashimoto's thyroiditis had strong intensity and rest 2 had moderate intensity. Among follicular adenoma 4 cases had moderate intensity while 1 case had mild intensity. All 15 cases of malignant papillary carcinoma and medullary carcinoma shows TPO negativity. The final immunohistochemical score for TPO marker showed strong positivity for multinodular goitre and hashimoto's thyroiditis and moderately positive for follicular adenoma and negative in case of malignant lesion. Out of 100 cases, all the 15 malignant thyroid lesion were positive for CK 19 marker and showed grade 4 staining, while among 85 benign cases 21 cases of multinodular goitre showed grade 1 staining of cell, 9 cases showed grade 2, and 1 cases showed grade 3 staining. Among 6 cases of hashimoto's thyroiditis 2 cases showed grade 1 staining and in 5 cases of follicular adenoma 2 cases showed grade 2 staining. Out of the 100 cases, all 15 malignant thyroid lesion showed strong intensity for CK 19 staining, while among 74 cases of multinodular goitre 31 cases showed mild intensity and 2 cases each of hashimoto's thyroiditis and follicular adenoma showed mild intensity. The final immunohistochemical score for CK 19 staining revealed strong positivity for malignant thyroid lesion and the benign thyroid lesion showed weak positivity. The sensitivity and specificity of TPO staining in detection of benign lesion were found to be 100 % and 100% respectively with 100% positive predictive value and 100% negative predictive value. The sensitivity and specificity of CK 19 in detection of malignant thyroid lesion were found to be 100 % and 58.82% respectively with 30.0% positive predictive value and 100% negative predictive

Table 2: Distribution of cases into benign and malignant category. Benign and malignant thyroid lesion

Nature	No of cases	Percentage
Benign	85	85%
Malignant	15	15%
Total	100	100%

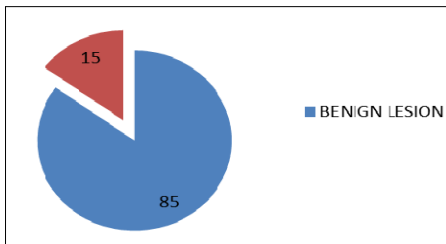


Fig 1: Benign and malignant thyroid lesion

Table 3: Age wise distribution of the cases studied

Age group (in years)	No. of cases	Percentage
21-30	07	07%
31-40	41	41%
41-50	42	42%
51-60	06	06%
60 above	04	04%
Total	100	100%

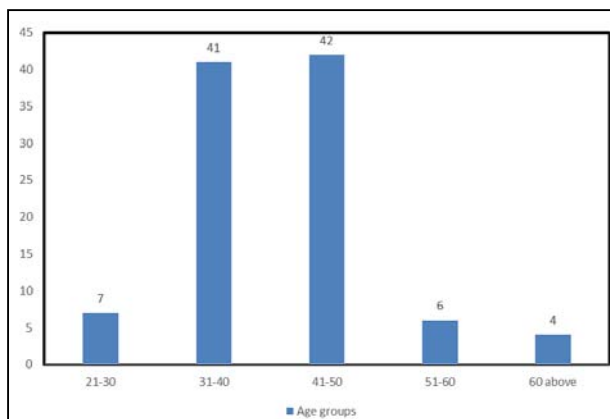


Fig 2: Age wise distribution of total cases

Table 4: Gender distribution of total cases studied

Gender	No. of cases	Percentage
Male	11.00	11.00%
Female	89.00	89.00%
Total	100	100%

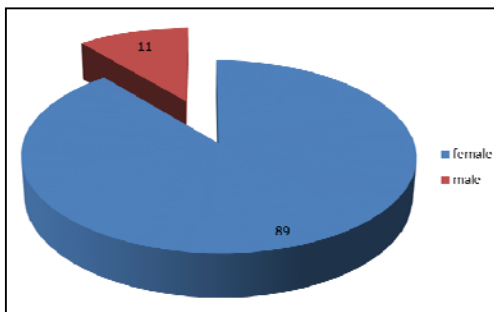


Fig 3: Gender wise distribution of thyroid lesions

Table 5: Statistical analysis of TPO in thyroid lesions

TPO Expression	Benign lesion	Malignant lesion	Total
	No.%	No.%	
Positive	85 (100%)	0 (0%)	85
Negative	0 (0%)	15 (100%)	15
TOTAL	85	15	100

Sensitivity	Specificity	Positive predictive value	Negative predictive value
1.000	1.000	1.000	1.000

Table 6: Statistical analysis of CK 19 in THYROID lesions

Expression of CK 19	Malignant lesion	Benign lesion	Total
	No. (%)	No. (%)	
Positive	15 (100%)	42 (35.70%)	50
Negative	0 (0%)	43 (36.55%)	50
TOTAL	15	85	100

Sensitivity	Specificity	Positive predictive value	Negative predictive value
1.000	0.5882	0.3000	1.000

Discussion

A total of 100 cases of formalin fixed paraffin embedded histological sections of various thyroid lesions, both benign and malignant were examined. Immunohistochemical staining of TPO and CK 19 was done to study and analyze their expression in various benign and malignant thyroid lesion.

In our study the most common age group was between 41-50 yrs. According to the the study done by Mahima Sharma *et al.* 2017 [20] it was observed that maximum number of thyroid cases were in age group of 41-50 years(20.69%) followed by cases in the age group of 31-40 years.(15.86%). In our study, nonneoplastic lesion were more common. Study done by Dr Mital j.Gamit *et al.* [21] observed that majority of cases were benign with 60% cases of goitre,10% cases of hashimotos thyroiditis,7.5% cases of follicular adenoma, 6.25% of papillary carcinoma while medullary carcinoma were seen in 1.25% cases.in our study female are affected more than male.. In our study, TPO expression was found to be 100% in non-neoplastic and benign lesions and its expression was completely loss in malignancy. The study done by De micco *et al.* [8] 1999 demonstrated that anti TPO antibody MoAb 47 expressed in all normal and benign thyroid lesions but only 3% of malignant lesions. Zeming liu *et al.* [19] 2015, reported that anti TPO antibody MoAb 47 expressed in all normal and benign thyroid lesions but only 12% of malignant lesions. The study done by Hemanathan *et al.*, [22] (2017) demonstrated that anti TPO antibody MoAb 47 expressed in all normal and benign thyroid lesions and absent in all of malignant lesions. In our study ck-19 immunoreactivity was found to be diffuse and strong in malignancy and pale and focally staining in benign conditions. The study done by Michel R Nasr *et al.* [23] 2006 found to express CK 19 in 50% of benign lesion and 100% in malignant lesions. The study done by Hemanathan *et al.* [22] 2017 found to express CK 19 in 48% of benign lesion and 100% in malignant lesions.

Immunohistochemical evaluation of TPO in benign and malignant thyroid lesion.

Table 7: Comparison analysis of tpo expression in thyroid lesions

Study	Benign lesion	Malignant lesion
De <i>et al.</i> ,	100%	3%
Zeming liu <i>et al.</i> ,	100%	12%
Lima <i>et al.</i> ,	100%	20%
S savin <i>et al.</i> ,	100%	21%
Hemanathan <i>et al.</i> , (2017) ^[17]	100%	0%
present study	100%	0%

Immunohistochemical evaluation of CK 19 in benign and malignant thyroid lesion.

Table 8: Comparative analysis of CK 19 expression in thyroid lesions.

Study	Benign lesion	Malignant lesion
Michel R Nasr <i>et al.</i>	50%	100%
Bose D <i>et al.</i>	50%	100%
Cheung <i>et al.</i>	20%	80%
Beesley <i>et al.</i>	25%	100%
Won young <i>et al.</i>	50%	90%
Hemanathan <i>et al.</i> ,	100%	100%
Present study	49.41%	100%

Our study shows that the sensitivity and specificity of TPO staining in detection of benign lesion were found to be 100 % and 100% respectively with 100% positive predictive value and 100% negative predictive value. Study done by of Hemanathan., *et al.* (2013) ^[22] showed that the sensitivity and specificity of TPO in detection of benign were 100% and 100% respectively. Positive predictive value (PPV) was 100%, while negative predictive value (NPV) was 100%. Our study shows the the sensitivity and specificity of CK 19 in detection of malignant thyroid lesion were found to be 100 % and 58.82% respectively with 30.0% positive predictive value and 100% negative predictive value. Study done by Hemanathan., *et al.* (2013) ^[22] concluded that the sensitivity and specificity of CK 19 in detection of malignant lesions were 100% and 0% respectively. Positive predictive value (PPV) was 24.61%, while negative predictive value (NPV) was 0%.

Limitation

There are few limitation in the present study.ck-19 expression was observed in benign thyroid lesion. Hence it is very important to use tpo along with ck-19 in such cases, leading to an increase in the cost of the test.

Conclusion

The results from our study implicates that a combination of both positive and negative marker is essential in distinguishing benign and malignant conditions. TPO expression indicates benign condition and loss of its expression indicates malignant transformation and strong membranous CK-19 positivity indicates malignancy. Thus gain of CK-19 expression and loss of TPO expression were both considered significant in the progression of thyroid carcinoma.

References

1. Anirban Maitra. The endocrine System: Pathologic basis of diseases. Eight ed; chapter 24, 1118-1120.
2. Mulazim Hussain, Bukhari Asad, Aslam Khan, Shahida Niazi, Madiha Arshad, Zahid Mahmood, *et al.* Better thyroid cytopathology Reporting system may increase the clinical management and patient outcome. J cytology and histology. 2012; 3:3-6.
3. Tan GH, Gharib H, Reading CC. Solitary thyroid nodule. Comparison between palpation and ultrasonography. Arch intern Med. 1995; 155:2418-2423.
4. Cooper DS, Doherty GM, Haugen BR, *et al.* Management guideline for patients with thyroid nodules and differentiated thyroid cancer. Thyroid. 2006; 16:109-142.
5. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973–2002. Journal of the American Medical Association. 2006; 295:2164-2167.
6. Beesley A, McLaren MF. Km: cytokeratin 19 +gelactin-immunohistochemistry in the d/d of solitary thyroid nodules. histopathology. 2002; 41:236-243.
7. Lima MA, Gontijo VA, Schmitt FC. Thyroid peroxidase and thyroglobulin expression in normal human thyroid glands. Endocr Pathol. 1998; 9:333-8.
8. Lima MA, Gontijo VA, Santos MC, Schmitt FC. Thyroid peroxidase expression in diseased human thyroid glands. Endocr Pathol. 1999; 10:223-8.
9. De Micco C, Vasko V, Garcia S, Zoro P, Denizot A, Henry JF. Fine-needle aspiration of thyroid follicular neoplasm: diagnostic use of thyroid peroxidase immunocytochemistry with monoclonal antibody 47. Surgery. 1994; 116:1031-5.
10. De Micco C, Zoro P, Garcia S, Skoog L, Tani EM, Carayon P, Henry JF. Thyroid peroxidase immunodetection as a tool to assist diagnosis of thyroid nodules on fine-needle aspiration biopsy. Eur J Endocrinol. 1994; 131:474-9.
11. Faroux MJ, Theobald S, Pluot M, Patey M, Menzes D. Evaluation of the monoclonal antibody antithyroperoxidase MoAb47 in the diagnostic decision of cold thyroid nodules by fine-needle aspiration. Pathol Res Pract. 1997; 193:705-2.
12. Lazar V, Bidart JM, Caillou B, Mahe C, Lacroix L, Filetti S, Schlumberger M. Expression of the Na+/I-symporter gene in human thyroid tumours: a comparison study with other thyroid-specific genes. J Clin Endocrinol Metab. 1999; 84:3228-34.
13. Christensen L, Blichert-Toft M, Brandt M, Lange M, Sneppen SB, Ravnsbaek J, *et al.* Thyroperoxidase (TPO) immunostaining of the solitary cold thyroid nodule. Clin Endocrinol. 2000; 53:161-9.
14. Gerard AC, Daumerie C, Mestdagh C, Gohy S, De Burbure C, Costagliola S, *et al.* Correlation between the loss of thyroglobulin iodination and the expression of thyroid-specific proteins involved in iodine metabolism in thyroid carcinomas. J Clin Endocrinol Metab. 2003; 88:4977-83.
15. De Micco C, Zoro P, Henry JF. Malignancy markers in the cytodagnosis of thyroid nodules. Thyroid peroxidase. Annals of Pathology. 1994; 14:383.
16. Nasr MR, Mukhopadhyay S, Zhang S, Katzenstein AL. Immunohistochemical markers in diagnosis of

- papillary thyroid carcinoma: utility of HBME1 combined with CK19 immunostaining. *Mod Pathol.* 2006; 19(12):1631-1637.
17. Baloch ZW, Abraham S, Roberts S, LiVolsi VA. Differential expression of cytokeratins in follicular variant of papillary carcinoma: an immunohistochemical study and its diagnostic utility. *Hum Pathol.* 1999; 30(10):1166-1171.
 18. Nasser SM, Pitman MB, Pilch BZ, *et al.* Fine-needle aspiration biopsy of papillary thyroid carcinoma: diagnostic utility of cytokeratin 19 immunostaining. *Cancer.* 2000; 90:307-311.
 19. William WD, Doniach I, *et al.* CK19 as a single marker is as high as 92% and 97% respectively, 1977.
 20. Zeming Liu, Pan Yu, *et al.* Significance of CK-19, TPO, and HBME-1 expression for diagnosis of papillary thyroid carcinoma. *Int J Clin Exp Med.* 2015; 8(3):4369-74.
 21. Mahima Sharma, Anuja Sharma, Shivani Gandhi, Arvind Khajuria, Goswami KC. Histopathological patterns of head and neck lesions -a two year retrospective hospital based study, 2017.
 22. Dr. Mital J Gamit, Dr Sanjay R Talwelkar, Dr. Gauravi A. Dhruva. Histochemical Correlation Study of Thyroid Gland Lesions.
 23. Hemanathan Guhanandam, Revathishree Rajamani, Naseen Noorunnisa, Manimaran Durairaj. Expression of Cytokeratin-19 and Thyroperoxidase in Relation to Morphological Features in Non-Neoplastic and Neoplastic Lesions of Thyroid.
 24. Nasr MR, Mukhopadhyay S, *et al.* Immunohistochemical markers in the diagnosis of papillary thyroid carcinoma: utility of HBME1 combined with CK-19 immunostaining. *Modern Pathology.* 2006; 19:1631-37.