



Original Article

Expression of CK-19 Immunomarker in Thyroid Neoplasm and its Association with Histopathology

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Abstract

Background & Objective: Early and accurate diagnosis of thyroid neoplasm is of utmost significance for prolongation of patient survival. A panel of immunomarkers has been tested to overcome the limitation of histopathology. CK-19 is a commonly used immunomarker among them. This study was undertaken to evaluate the diagnostic accuracy of CK-19 in thyroid neoplasm.

Methods & Materials: This cross-sectional study was conducted in the Department of Pathology, Rajshahi Medical College, over a period of two years, from July 2017 to June 2019. A total of 44 tissue blocks of histologically confirmed thyroid neoplasm were included in this study. Then immunohistochemistry was done for CK-19 from a significant paraffin-embedded block.

Result: In the present study, diffuse and strong positivity of CK-19 was found in papillary thyroid carcinoma (PTC), followed by focal positivity in follicular (FC) and medullary carcinoma (MC). A negative staining pattern was found in all cases of follicular adenoma (FA). The sensitivity and specificity of CK-19 were found to be high in the case of PTC, respectively, 88% and 92%. CK-19 expression was highly specific in differentiating malignant from benign thyroid tumors (100%).

Conclusion: The study concluded that CK 19 is a highly sensitive and specific marker in papillary thyroid carcinoma. Diffuse and strong positivity is characteristic of PTC, which can be used in diagnosis with equivocal morphological appearance.

Keywords: Thyroid Neoplasm, Immunomarker, CK-19, Papillary thyroid carcinoma.

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Introduction

The thyroid is the most common endocrine gland to undergo neoplastic transformation. Nowadays,

the thyroid tumors account for 1.5% of all cancers in adults in Bangladesh.¹ If the recent trend is

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maintained, thyroid cancer may become the fourth most common cancer by 2030.²

Early diagnosis of thyroid tumors and appropriate management will prolong the survival rate of patients. As many thyroid tumors have overlapping morphological features, an exact diagnosis is essential for patients' surgical and post-operative management. Especially papillary carcinoma and its follicular variant, which mimics follicular carcinoma, can be treated by simple thyroidectomy if diagnosed early. Some benign tumors or other non-neoplastic lesions present with focal papillary hyperplasia, which also misguide the diagnosis. Differentiation of follicular adenoma and follicular carcinoma depends on capsular and vascular invasion. When it is inconclusive, a false diagnosis of benignity may lead to extensive vascular dissemination and a dismal prognosis.³

The gold standard for the diagnosis of thyroid neoplasm is histopathology till now. However, distinguishing various thyroid lesions by hematoxylin and eosin sections alone is really challenging for the pathologist. Immunohistochemistry (IHC) offers an advantage in the case where histomorphological details are equivocal and insufficient to establish a definitive diagnosis. In such a case, immunomarker cytokeratin-19 (CK-19) expression in the diagnosis of thyroid neoplasm will accomplish that goal.⁴

Cytokeratin 19, a low molecular weight protein of 40k Da belonging to the keratin family, is an intermediate filament involved in protein binding and organizing of myofibers. Healthy thyroid follicular cells do not produce this protein, and the upregulation of CK-19 is connected with neoplastic transformation.⁵

In recent years, few studies have demonstrated the clinical significance of CK-19 expression in various thyroid tumors. A strong and diffuse staining pattern of CK-19 had been shown in the case of Papillary thyroid carcinoma, both in classical and follicular variants, as well as variable focal immunoreactivity in other thyroid malignancies.⁶ Thus, the immunoreactivity and

distribution pattern of CK-19 will be a crucial marker along with histopathology to give an accurate interpretation of thyroid neoplasm.⁷ So, this study will overcome the limitations of histopathology and will help in proper diagnosis, management, and outcome for the patients.

Materials and Methods

This cross-sectional study was conducted in the Department of Pathology, Rajshahi Medical College, over a period of two years, from July 2017 to June 2019. A total of 44 tissue blocks of histologically confirmed thyroid neoplasm were included in the study. A purposive method of sampling was employed. The history of the patients was obtained from the hospital records. Specimen of tissue was fixed with 10% formalin and stained with hematoxylin, and eosin stain was examined. Then immunohistochemistry was done from a significant paraffin-embedded block with a monoclonal antibody against CK-19 antigens in appropriate dilutions. Positive staining for CK-19 pertained to diffuse or focal and cytoplasmic +/- membranous. The intensity of positivity will be graded from 0 to 4+ as follows-

Serial No.	Grading	Percentage of cells expressing CK19 positivity
1.	0	No positively staining cells
2.	1+	<5% of positively staining cells
3.	2+	5 – 24% of positively staining cells
4.	3+	25 – 75% of positively staining cells
5.	4+	>75% of positively staining cells

Staining of 1+ is defined as focal positive, 2+ is defined as moderate staining, and staining of 3+ or 4+ is defined as diffuse positive.⁸

Collected data were processed and analyzed with the help of SPSS (Statistical Package for Social Sciences), version 25. The diagnostic accuracies of CK-19 are determined by comparing the findings of these diagnostic modalities. The sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of the test were computed. The level of significance was set at 5%, and a p-value < 0.05 was considered significant.

Results

A total of 44 cases of histologically diagnosed thyroid neoplasm, either benign or malignant, were subjected to the immunohistochemical examination of CK-19.

Table I: Frequency of different histologic types of thyroid neoplasm (n=44)

Histological diagnosis	No of cases	Percentage (%)
Follicular Adenoma	21	47.7
Papillary Carcinoma		
Classic variant	15	34.3
Follicular variant	02	4.5
Follicular Carcinoma	04	9.0
Medullary Carcinoma	02	4.5
Total	44	100

Among the 44 cases, the highest number of cases were diagnosed as a follicular adenoma (21 cases, 47.7%), followed by papillary carcinoma (17 cases, 38.8%). The number of follicular carcinomas and medullary carcinoma were (4 cases, 9%) and (2 cases, 4.5%), respectively. 2 of the papillary carcinomas are follicular variants.

Table II: Intensity of staining of cytokeratin 19 in thyroid neoplasm (n=44).

Intensity of CK-19	FA	PTC (classic variant)	FVPC	FC	MC	Total
0	21	1	1	3	1	27
1+	0	0	1	1	1	03
2+	0	4	0	0	0	04
3+	0	5	0	0	0	05
4+	0	5	0	0	0	05
Total	21	15	2	4	2	44

In follicular adenoma, all cases were negative for CK-19. In this present study, the staining intensity of CK-19 in classic papillary carcinoma showed strong and diffuse positivity in the cytoplasm of cells, i.e., 3+ or 4+ staining in most of the cases comprising 76%. In the follicular variant of papillary carcinoma, 50% of cases showed 1+ positivity, and 50% of cases had negative expression. In follicular carcinoma, CK-19 was focally positive, i.e., 1+ in only 1 case comprising (25%). The rest 4 of the cases (75%) are negative for CK-19. In medullary carcinoma, 50% of cases showed 1+ positivity, and in the rest, 50% were negative (50%). None of the cases other than papillary thyroid carcinoma had been shown diffuse or strong positivity of CK-19.

Table III: Statistical analysis data of CK-19 in different thyroid neoplasm (n=44).

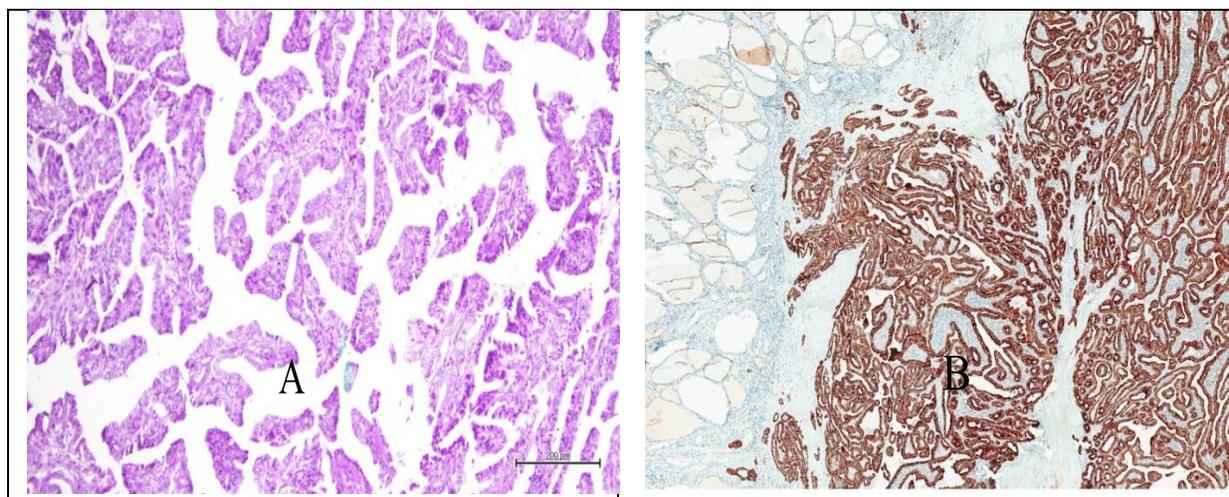
Thyroid tumors	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
PTC	88.24%	92.60%	88%	92%	91%
FC	25%	60%	5.88%	89%	56.82%
MC	50%	62%	5.88%	96.30%	61%
FA	05%	26%	06%	22.22%	13.64%

From the statistical analysis in this present study, it was found that CK-19 is a highly sensitive and specific marker in the diagnosis of papillary carcinoma. The diagnostic accuracy of CK-19 was relatively high in all varieties of thyroid carcinoma.

Table IV: Accuracy of CK-19 in differentiating malignant from benign thyroid tumors (n=44).

CK-19 expression	Histopathological diagnosis		Total
	Malignant	Benign	
Positive	17	00	17
Negative	06	21	27
Total	23	21	44

Table IV showed the accuracy of CK-19 as a positive marker in diagnosing thyroid tumors. The sensitivity of CK-19 in correctly differentiating malignant thyroid tumors from benign ones was $17/23 \times 100 = 74\%$, while the specificity of the test incorrectly excluding those who did not have malignancy was $21/21 \times 100 = 100\%$. The positive and negative predictive values of the test were $17/17 \times 100 = 100\%$ and $21/27 \times 100 = 77.78\%$, respectively. The overall diagnostic accuracy of the test was $(17 + 21)/44 \times 100 = 86.36\%$. The Chi-square test showed a statistically significant p-value of <0.05 from the above variables.



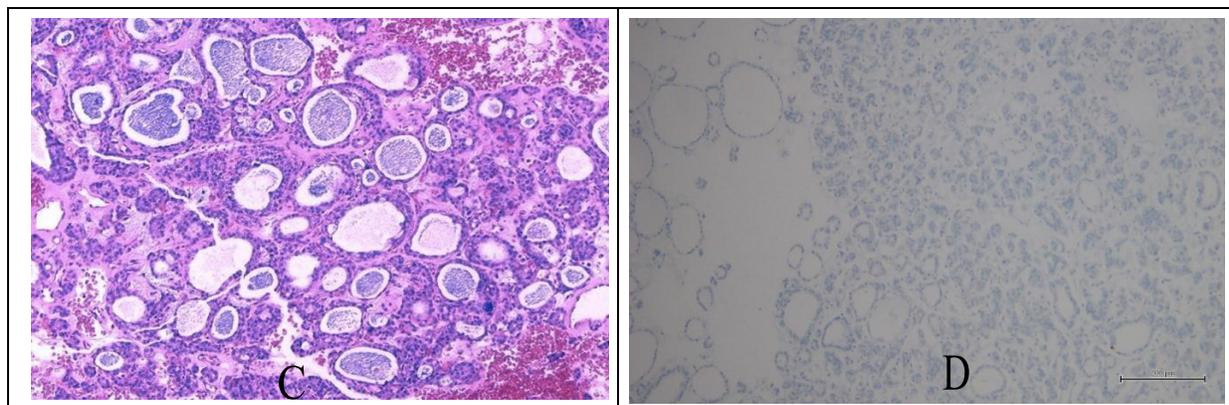


Figure I. A. Microscopic picture of papillary thyroid carcinoma, B. Diffuse & strong CK-19 expression in papillary thyroid carcinoma, C. Microscopic picture of follicular adenoma, D. Negative CK-19 expression in follicular adenoma.

Discussion

In this study, an attempt was made to assess the role of CK-19 as a sensitive marker in the diagnosis and also to differentiate various subtypes of thyroid neoplasm.

National Cancer Institute, 2013 analyzed the proportion of various thyroid carcinoma and found that papillary thyroid carcinoma is the predominant malignancy constituting 70- 80% of all thyroid cancers. The next common malignancy is follicular carcinoma constituting 10- 15% of all thyroid malignancies. In this present study, it was also obtained similar results, with papillary carcinoma constituting 76%, followed by follicular carcinoma at 16%.⁹

As the findings of CK-19 expression of the study were compared and contrasted with those of other studies conducted around the world, a similar picture was evident. In our present study, negative staining of CK-19 was found in all cases of follicular adenoma, as shown in Table II. We obtained focal and weak (1+) positivity in only one case out of a total of 4 cases of follicular carcinoma, constituting 25%. The rest of the 4 cases were negative, as shown in Table II. The sensitivity and specificity of CK-19 in follicular carcinoma in this study were 25% and 60%, respectively. This result was similar to other studies.^{10,11}

Hanan Alsaeid Alshenawy, stated in his study that CK-19 is always positive in papillary carcinoma in contrast to other thyroid malignancies. He reported that all 14 cases of papillary carcinoma showed positivity with cytokeratin 19 comprising 100%. 13 cases showed strong and diffuse positivity of 3+, and only one case showed 2+ intensity. In the follicular variant of papillary carcinoma, all 8 cases showed positive staining, with 5 cases showing 2+ intensity and the remaining 3 cases with 3+ positivity. He also found that CK-19 has 100% sensitivity and 77% specificity in differentiating papillary thyroid carcinoma from other follicular neoplasms. Similar findings were found by Kaliszewskiet *al.*, in 2016, Dunderovic *et al.*, in 2015, and Songet *al.*, in 2011.^{5,12-14}

In this present study, the results obtained were so similar to all the aforementioned studies. We studied the CK-19 staining in 88% of papillary carcinoma, including the follicular variant. Out of the 17 cases, positive and diffuse staining of CK-19 showed in 15 cases. In the total of 2 cases of follicular variant of papillary, one showed 1+ intensity, and the other showed negative staining as in table II. The sensitivity and specificity of CK-19 in papillary carcinoma are 88% and 92%, respectively.

Palo S in 2017 found negative staining of CK-19 in medullary carcinoma. The result was not similar to this study. In this study, focal or weak positivity was seen in one case out of two.⁴

The sensitivity and specificity of CK-19 expression in differentiating malignant tumors from benign were 74% and 100%, respectively. The sensitivity was quite similar to another study(75%).⁴ But specificity and diagnostic accuracy is relatively high than others which were 100% and 86%, respectively. Summarizing the findings of the present study and those of other investigators compared and contrasted, it is evident that CK-19 is a highly sensitive marker in the case of diagnosis of papillary carcinoma in the thyroid.

Conclusion

CK-19 expression estimation plays an active and complementary role in the accurate diagnosis of thyroid neoplasm when the tumor exhibit equivocal histomorphologic features. CK-19 showed 88% positivity with pattern provided the staining is strong and diffuse in papillary carcinoma and is found to be a sensitive and specific marker in the diagnosis of papillary carcinoma and its variants. So, this study can be concluded that CK-19 is a sensitive, specific, and positive diagnostic marker in thyroid neoplasm, especially papillary thyroid carcinoma.

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Conflict of interest: None declared

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