Immunohistochemical Expression of Cytokeratin-19 in Thyroid Nodules and Its Correlation With Histopathology

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ABSTRACT

Background: Thyroid neoplasms constitute the most commonly occurring endocrine tumors worldwide. They commonly occur between 30-60 years of age and affect both sexes. Papillary carcinoma is the most common malignant tumor constituting 80-85% of all the thyroid carcinomas followed by follicular carcinoma comprising 10-15%. Diagnosis becomes challenging when typical features are not seen or when overlapping features are present. In such cases immunohistochemistry can be done to make correct diagnosis.

Methods: In this study we observed the immunohistochemical expression of Cytokeratin19 in 30 thyroidectomy specimens which included classic papillary carcinoma (11 cases), follicular variant of papillary carcinoma (2 cases), follicular adenoma (15 cases) and follicular carcinoma (4 cases). The study period was one year. The scoring was given from 0- 3+ based on the percentage of cells showing membrane and cytoplasmic positivity and intensity of staining.

Result: The staining results of Cytokeratin19 showed diffuse and strong 3+ positivity in 8/11 cases of papillary carcinoma and its follicular variant, 2 cases showed 2+ positivity and one case with 1+ positivity. Majority of the cases of follicular adenoma (13/15 cases) and follicular carcinoma (3/4 cases) showed negative staining. Only 2 cases of follicular adenoma and 1 case of follicular carcinoma showed focal weak positivity.

Conclusion: Cytokeratin19 was found to be a sensitive and specific marker in diagnosing papillary carcinoma and its follicular variant. But it does not differentiate follicular neoplasms. Hence a good panel of markers is essential in arriving at exact diagnosis of thyroid neoplasms in challenging cases.

Keywords: Papillary Carcinoma, Follicular Adenoma, Follicular Carcinoma, Cytokeratin19.
• Follicular adenoma
• Follicular carcinoma.
• Papillary carcinoma (one with lymph node metastasis)
• Follicular variant of papillary carcinoma.

Follicular carcinoma is differentiated from follicular adenoma by widespread invasion into thyroid parenchyma, capsular invasion and/or vascular invasion. These tumors may lack complete encapsulation and may present with distant metastasis at the initial presentation. [4, 5]

Papillary carcinoma is diagnosed based on papillary pattern and characteristic nuclear features like ovoid ground glass nuclei with fine chromatin, nuclear grooves, nuclear pseudoinclusions, nuclear crowding and overlapping with or without psammoma body. [6]

IHC was done by utilizing a monoclonal anticytokeratin 19 antibody (clone -RCK 108), a mouse monoclonal antibody. Skin samples were used as positive control. The antigen retrieval was done by microwave method using citrate buffer at pH 6. Diaminobenzidine tetrachloride (DAB) was used as a chromogen and hematoxylin as a counterstain.

Positive staining refers to diffuse staining of membrane and cytoplasm. Intensity of positive staining was graded from 0 to 3+. Scoring was done based on the intensity of staining and percentage of cells expressing cytokeratin19 as shown below. [7]

| S.No | Grading         | Percentage of cells expressing CK19 positivity               |
|------|----------------|------------------------------------------------------------|
| 1.   | 0 (negative)   | No positively staining cells                                |
| 2.   | 1+ (focally positive) | <25% of positively staining cells                       |
| 3.   | 2+ (positive)  | 25 – 50% of positively staining cells                     |
| 4.   | 3+ (diffusely positive) | >50% of positively staining cells                      |

Results

A total of 30 cases of thyroidectomy specimens were taken for this study. Histomorphological features and immunohistochemical expression pattern of cytokeratin19 in thyroid nodules were studied, analysed and compared with literature.

In the present study the age distribution of thyroid neoplasms was most common in the age group between 41-50 years, comprising about 40% (12 cases) of the total cases (Table 1). Thyroid nodules were more common among females constituting about 90% (27 cases out of 30). Female to male ratio was 3:1 in this study.

Comparing the incidence of different thyroid neoplasms, follicular adenoma constituted the majority of cases (50%), followed by papillary carcinoma (37%- classic type-30%, follicular variant- 7%) and follicular carcinoma -13% (Table 2).

The intensity of staining of cytokeratin19 in various thyroid neoplasms were analysed and tabulated (Table 3, chart 1). In the present study, staining intensity of cytokeratin19 in classic papillary carcinoma showed strong and diffuse positivity in membrane and cytoplasm of cells i.e. 3+ staining in 8cases comprising 89% , with only 11% of cases showing moderate or 2+ staining (Fig 1, Fig 2). In follicular variant of papillary carcinoma out of 2 cases, one showed 2 + positivity (Fig 3), other case showed 1 + positivity Thus all cases of papillary carcinoma showed cytokeratin19 expression i.e. 100%.

In follicular carcinoma cytokeratin19 is weakly positive i.e. 1+ in only one case while other cases were negative for cytokeratin19 (Fig 4).

In follicular adenoma 1+ weak and focal positivity was obtained in 13% of cases only (Fig 5). 87% of cases were negative for cytokeratin19.

Chi square test showed statistically significant p value of <0.001 from the above variables.

| Table 1 |
|---------|
| Age in years | No. of cases | Percentage |
| < 30      | 8            | 27%        |
| 31 – 40   | 8            | 27%        |
| 41 – 50   | 12           | 40%        |
| 51 – 60   | 2            | 6%         |
| Total     | 30           | 100%       |

| Table 2: distribution of different thyroid neoplasms: |
|----------|
| Histopathological Diagnosis | No. of cases | Percentage |
| Papillary Carcinoma         | 11           | 37%        |
| Classic type                | 9            | 30%        |
| Follicular variant           | 2            | 7%         |
| Follicular Carcinoma        | 4            | 13%        |
| Follicular Adenoma          | 15           | 50%        |
TABLE 3: Scoring Of Cytokeratin19 Expression In Thyroid Nodules.

| Staining of CK 19 | HPE DIAGNOSIS |
|-------------------|---------------|
|                   | PC | FC | FA | PCFV | TOTAL |
| 0                 | 0  | 3  | 13 | 0    | 16    |
| 1+                | 0  | 1  | 2  | 1    | 4     |
| 2+                | 1  | 0  | 0  | 1    | 2     |
| 3+                | 8  | 0  | 0  | 0    | 8     |
| **Total**         | 9  | 4  | 15 | 2    | 30    |

(Note: 0: no cells are positive; 1+: <25% of cells are positive; 2+: 25%-50% of cells are positive; 3+: >50% of cells are positive, PC: Papillary carcinoma, PCFV: Papillary carcinoma - Follicular variant, FA: Follicular adenoma, FC: Follicular Carcinoma, HPE: Histopathology)

TABLE 4: Statistical Analysis Datas Of Cytokeratin19 Staining In Thyroid Nodules.

| Thyroid tumors                  | Sensitivity | specificity |
|---------------------------------|-------------|-------------|
| Classic papillary carcinoma     | 100%        | 84%         |
| Papillary carcinoma-Follicular variant | 100%        | 57%         |
| Follicular carcinoma            | 25%         | 50%         |
| Follicular adenoma              | 13%         | 87%         |

TABLE 5: Comparison Of Results Of Cytokeratin19 Expression In Various Studies With Our Study.

| Study name                  | Percentage of cases with positive cytokeratin19 expression |
|-----------------------------|----------------------------------------------------------|
|                             | Follicular adenoma | Follicular carcinoma | Papillary carcinoma and follicular variant |
| Hanan Alsaeid Alshenawy     | 57%               | 53%                   | 100%                                     |
| Husain A Saleh et al        | 30%               | -                     | 85%                                      |
| K.Y Lam et al               | -                 | 68%                   | -                                        |
| Dina El Demellawy et al     | -                 | -                     | 85%                                      |
| Our study                   | 13%               | 50%                   | 100%                                     |

Fig. 1: Diffuse 3+ positivity of cytokeratin19 in papillary carcinoma (10X).

Fig. 2: 2+ positivity of Cytokeratin19 in papillary carcinoma (10X)
Fig. 3: Follicular variant of papillary carcinoma showing 2+ positivity with cytokeratin19 (10X).

Fig. 4: Follicular carcinoma showing negative staining with cytokeratin19.

Fig. 5: Follicular adenoma showing focal 1+ positivity with cytokeratin19 (10X).

Fig. 6: Diffuse 3+ positivity of cytokeratin19 in metastatic papillary carcinoma deposits in lymph node (10X).

Chart 1: Scoring of Cytokeratin19 Expression in Thyroid Nodules
Discussion

Prevalence of thyroid nodules steadily increases with age. Presence of solitary nodule, a multinodular gland and one or more cystic lesions indicate nodular thyroid diseases. [8] But 80% of the thyroid nodules are benign or hyperplastic nodules. Thyroid malignancies are the most common endocrine carcinomas comprising 5-10%. [9] Diagnosis of thyroid lesions is difficult when multiple different architectural patterns are present within the same tumor. In addition, encapsulated tumors with follicular pattern pose dilemma in differentiating benign from malignant thyroid neoplasm, when the neoplastic nodule has incomplete capsular invasion or equivocal vascular invasion. [9] Follicular neoplasms/ atypical cells of undetermined significance is yet another category which accounts for 10-25% of all cases and represents a therapeutic problem because of low risk of malignancy. [10] In view of all these shortcomings, nowadays a panel of immunohistochemical markers are used that help in the distinction of benign and malignant thyroid lesions. Many studies have been done using various immunomarkers to distinguish thyroid nodules but their role in diagnosis is still questionable. In this present study, the usefulness of cytokeratin19 in the differential diagnosis of various thyroid nodules has been analysed and correlated with histomorphology.

Alshenawy et al observed focal and weak cytokeratin 19 positivity in 57% of follicular adenoma i.e. 4 cases out of total 7 cases in his study. [7] Husain A Saleh et al, in their study reported cytokeratin19 positivity in 30% (23 cases out of 46 total cases) of cases of follicular adenoma and the intensity of staining is weak and focal. [9] In our present study, the results were similarly weak and focally positive with cytokeratin19 i.e. 1+ in 2 cases out of total 15 cases, constituting 13%. All other 13 cases showed negative staining. Hence cytokeratin19 is not a sensitive marker in the diagnosis of follicular adenoma. Lam et al, reported focal positivity in 68% of follicular carcinoma, 13 cases out of total 19 cases with cytokeratin19. [11]

In this present study we obtained focal 1+ positivity in only 1 case out of total 4 cases of follicular carcinoma constituting 25%. The sensitivity and specificity of cytokeratin19 in follicular carcinoma in this study was 25% and 50% respectively. Demellawy et al observed, cytokeratin19 positivity in 85% of cases of papillary carcinoma which included both classic and follicular variant. [12]

We studied the cytokeratin19 staining in total of 11 cases of papillary carcinoma including follicular variant. All the 11 cases showed positive staining (100%) with cytokeratin19 but with varying intensity. 8 cases expressed diffuse and strong positivity of 3+ (89%) in classic papillary carcinoma. Only one case (11%) showed 2+ intensity of staining.

One of the above cases of classic papillary carcinoma had cervical lymph node metastasis and cytokeratin19 showed diffuse and strong 3+ positivity (Fig 6). Among the 2 cases of follicular variant of papillary carcinoma one showed 1+ score and other had score 2+. Similar results were observed in a study by Saleh et al i.e 85% and Alshenawy found 100% positive expression of cytokeratin 19 in papillary carcinoma and its follicular variant. [9, 7]

The sensitivity and specificity of cytokeratin19 in papillary carcinoma in our study was 100% and 84% respectively. Chi square test of these variables showed statistically significant p value of <0.001 (Table 4).

The results of this present study are in conformity with the previously conducted studies (Table 5) and thus immunomarker cytokeratin19 plays a pivotal role in the differentiation of thyroid nodules.

Conclusion

From this study cytokeratin19 is found to be a specific and sensitive (100%) marker for papillary carcinoma of thyroid and its follicular variant. The role of this marker in all other variants of papillary carcinoma has to be assessed. The identification of a specific marker for follicular neoplasms is crucial as using a panel of markers can turn out to be a cost effective test for the patient.

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