

## A Cross Sectional Study of Expression of Cytokeratin 19 in Papillary Thyroid Carcinoma and Its Association with Prognostic Factors

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### Abstract

**Introduction:** Thyroid malignancies are the most common endocrine malignancy. The most common malignancy in thyroid is papillary thyroid carcinoma. About 80% of all thyroid cancers are papillary carcinoma thyroid. Papillary carcinoma typically arises as an irregular, solid or cystic mass that comes from otherwise normal thyroid tissue. Papillary carcinoma has a high cure rate with 10-year survival rates for all patients with papillary carcinoma thyroid estimated at 80% to 90%. **Materials and Methods:** This cross-sectional study was conducted from January 2020 to December 2020. The cases included for the study were histopathologically proven cases of papillary thyroid carcinoma and its variants from thyroidectomy specimens received in Histopathology Department. Cases without proper data were excluded. It was a record-based study. Sample size =108. We took 160 samples for the study. **Results:** Of the total 160 cases of PTC, lymph node metastasis was noted in 38 cases that constituted 23.8 %, of which 2 cases was follicular variant of PTC and all others were classic variant of PTC. Out of 160 cases of PTC, 158 cases (98.8 %) showed 3+ or 4+ cytoplasmic and membranous positivity for CK19 and only 2 case showed a negative expression. 10 out of 24 cases of follicular variant showed a 3+ positivity, whereas columnar cell variant, tall cell variant and Warthin like variant showed 4+ positivity. **Conclusion:** Through this study it is concluded that, both CK-19 and CD56 can be used together as markers for PTC including all its variants but CK-19 cannot be used to assess the prognosis of papillary thyroid carcinoma as no association was identified in the study between the expression of CK-19 and prognostic factors.

**Keywords:** Thyroid malignancies, endocrine malignancy, CK-19, CD56.

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### Introduction

Thyroid malignancies are the most common endocrine malignancy. The most common malignancy in thyroid is Papillary thyroid carcinoma. About 80% of all thyroid cancers are papillary carcinoma thyroid[1]. Papillary carcinoma typically arises as an irregular, solid or cystic mass that comes from otherwise normal thyroid tissue. Papillary carcinoma has a high cure rate with 10-year survival rates for all patients with papillary carcinoma thyroid estimated at 80% to 90%.

CD56 is a neural cell adhesion molecule (NCAM), which has a role in cell adhesion. CD56 is present on follicular epithelial cells of normal thyroid and show diffuse membranous positivity. Previous studies show low or absent expression in all variants of papillary carcinoma thyroid.

Most common subtypes of PTC include the classic, follicular and tall cell variants. Many other uncommon variants like oncocytic, columnar cell, diffuse sclerosing and solid forms, clear cell and cribriform morular variant have also been described. There are many clinical and pathological factors that are associated with the poor prognosis of papillary carcinoma, which includes age, gender, distant metastasis, size of the primary tumour, subtypes and lymph node involvement.

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Our objective was to assess the expression of CK19 in papillary thyroid carcinoma and to find its association with few of these prognostic parameters like age, gender, tumour size and metastasis to lymph nodes. Also, to study the expression of CD56 in papillary thyroid carcinoma. The association between CK19 expression and the patient's unfavourable prognostic factors could suggest its possible role as a marker of poor prognosis of PTC.

### Materials and Methods

This cross-sectional study was conducted from January 2020 to December 2020. The cases included for the study were histopathologically proven cases of papillary thyroid carcinoma and its variants from thyroidectomy specimens received in Histopathology Department. Cases without proper data were excluded. It was a record-based study. Sample size =108. We took 160 samples for the study.

**Sampling Method:** First 160 consecutive specimens meeting the inclusion and exclusion criteria during the period of analysis were included in the study.

### Study Tool

1. Instruments to take bits of tissue to be studied.
2. Reagents for tissue processing.
3. Instruments for making paraffin blocks and cutting thin sections from it.
4. Haematoxylin and eosin (H & E) stains.
5. Glass slides and cover slips for mounting.
6. Microscope.
7. IHC markers CD56 and CK-19.
8. A structured proforma to record patient details: name, age, sex, inpatient number, histopathology report number, type of surgery, tumour size and metastasis and diagnosis made.

### Data collection technique

Structured proforma was used to collect data of the selected cases and its prognostic factors. Surgically resected thyroid tissues were fixed in 10 % neutral buffered formalin. Specimens were then grossed, and adequate samples were taken from appropriate area and then processed in automated tissue processing unit. After processing, tissues are embedded in paraffin wax and are then made into blocks for taking sections. Sections of 4 -5 micrometre thickness are cut from the blocks and stained with haematoxylin and eosin staining. The slides are analysed under optical microscope and diagnosis were entered in the proforma. Blocks of those slides showing tumour were collected. Unstained slides are made from them. Thinner sections are taken on aminopropyltriethoxysilane (APES) coated slides and incubated overnight for adequate fixation. Immunostaining for CD56 and CK-19 was done in our immune histochemistry laboratory following standard IHC protocol. The stained slides were then mounted and labelled. Interpretation of CK-19 and CD56 were done and results entered as positive or negative. The procedures and evaluation were done by single observer.

**Statistical Analysis:** All collected data was entered in Microsoft Excel sheet with all relevant details and analysed using statistical software SPSS version 23.0. The qualitative variables were expressed in percentage. The association was tested using appropriate statistical test.

### Results

The age ranges from 7 years to 75 years. 47 cases were in the age group less than or equal to 45 years and 33 cases were in the age group above 45 years. Higher percentage of papillary carcinoma was identified in the age group less than 45 years (58.8 %). Among 80 cases, 69 were female patients and 11 were males, with a female predominance of 86.3 %.

The variants of papillary carcinoma thyroid included were classic variant PTC, follicular variant PTC, tall cell variant, columnar cell

variant and Warthin like variant. Of which, classic variant predominates with a percentage of 81.3 %. 24 cases of follicular variant PTC were collected constituting 15 % and one case each for tall cell, columnar cell and Warthin like variants constituting 1.3 % each.

Comparing the size of PTC, the cases were grouped into  $\leq 1$  cm and  $>1$  cm. Majority of cases were more than 1 cm that accounts for 85 % of cases.

Of the total 160 cases of PTC, lymph node metastasis was noted in 38 cases that constituted 23.8 %, of which 2 case was follicular variant of PTC and all others were classic variant of PTC.

Out of 160 cases of PTC, 158 cases (98.8 %) showed 3+ or 4+ cytoplasmic and membranous positivity for CK19 and only 2 case showed a negative expression. 10 out of 24 cases of follicular variant showed a 3+ positivity, whereas columnar cell variant, tall cell variant and Warthin like variant showed 4+ positivity.

Of the 160 cases of papillary thyroid carcinoma, 154 cases were negative for CD56 expression that accounts for 96.3 %. 6 cases showed a 2+ positivity, but still there was a loss of expression when compared to the surrounding normal thyroid. We observed a diffuse CD56 staining reaction with distinctive membranous pattern in the normal thyroid.

The association between CK-19 expression and age of the patients was studied. Out of 94 cases whose age is less than or equal to 45 years, 92 cases showed 3+ or 4+ positivity for CK-19 (97.9 %) and 2 case showed a negative expression. And all the 66 cases who were in the age group above 45 years also showed positive expression for CK-19 (100 %). (Table 1) CK-19 expression and age of patients does not have a statistically significant relationship as the P-value is  $> 0.05$  (0.587)

**Table 1: Comparison of Age Based on CK-19 Expression**

| Age             | Positive |         | Negative |         | P Value |
|-----------------|----------|---------|----------|---------|---------|
|                 | Count    | Percent | Count    | Percent |         |
| $\leq 45$ years | 92       | 97.9    | 2        | 2.1     | 0.587   |
| $>45$ years     | 66       | 100.0   | 0        | 0.0     |         |

**Table 2: Comparison of Gender Based on CK-19 Expression**

| Gender | Positive |         | Negative |         | P Value |
|--------|----------|---------|----------|---------|---------|
|        | Count    | Percent | Count    | Percent |         |
| Female | 136      | 98.6    | 2        | 1.4     | 0.862   |
| Male   | 22       | 100.0   | 0        | 0.0     |         |

**Table 3: Comparison of Size Based on CK-19 Expression**

| Size        | Positive |         | Negative |         | P Value |
|-------------|----------|---------|----------|---------|---------|
|             | Count    | Percent | Count    | Percent |         |
| $\leq 1$ cm | 24       | 100.0   | 0        | 0.0     | 0.850   |
| $>1$ cm     | 134      | 98.5    | 2        | 1.5     |         |

**Table 4: Comparison of Lymph Node Metastasis Based on CK-19 Expression**

| Lymph Node | Positive |         | Negative |         | P Value |
|------------|----------|---------|----------|---------|---------|
|            | Count    | Percent | Count    | Percent |         |
| Present    | 36       | 94.7    | 2        | 5.3     | 0.237   |
| Absent     | 122      | 100.0   | 0        | 0.0     |         |

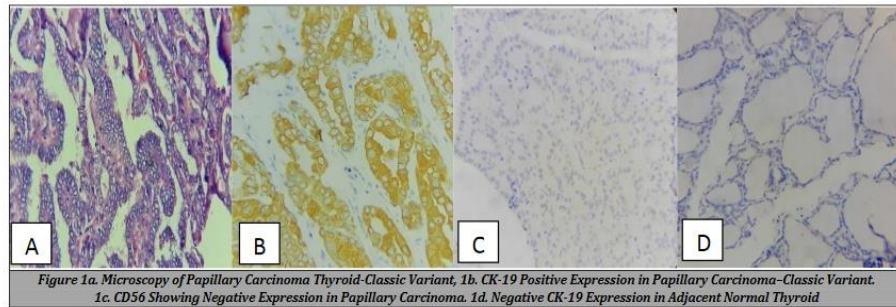


Figure 1a. Microscopy of Papillary Carcinoma Thyroid-Classical Variant. 1b. CK-19 Positive Expression in Papillary Carcinoma-Classical Variant. 1c. CD56 Showing Negative Expression in Papillary Carcinoma. 1d. Negative CK-19 Expression in Adjacent Normal Thyroid

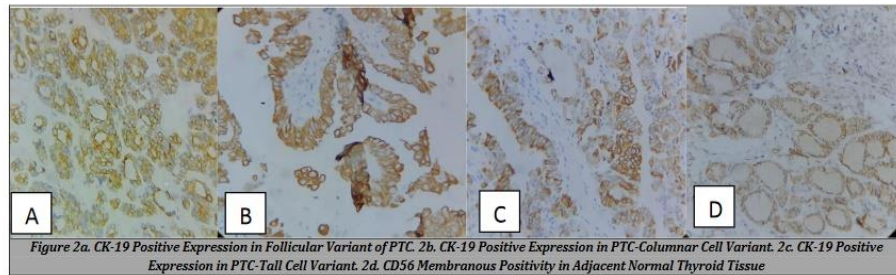


Figure 2a. CK-19 Positive Expression in Follicular Variant of PTC. 2b. CK-19 Positive Expression in PTC-Columnar Cell Variant. 2c. CK-19 Positive Expression in PTC-Tall Cell Variant. 2d. CD56 Membranous Positivity in Adjacent Normal Thyroid Tissue

The association between CK-19 expression and gender of the patients was studied. Out of 138 females, 136 cases (98.6 %) showed positivity for CK-19 expression and 2 cases (1.4 %) was negative. All the 22 cases who were male patients were positive (100 %) for CK-19 expression. From the statistical analysis, CK-19 expression and gender of the patient does not have a statistically significant relationship as the P-value was  $>0.05$  (0.862) (Table 2). The association between tumour size and CK-19 expression was studied. Of the 136 cases which was more than 1cm in size, 134 cases (98.5 %) showed positivity for CK-19 expression and 2 case showed a negative expression. All the 24 cases of PTC which was less than or equal to 1cm in size showed positive CK-19 expression (100 %). CK-19 expression and size of the tumour does not have a statistically significant relationship as the P-value is  $>0.05$  (0.850) (Table 3). The association between CK-19 expression and lymph node metastasis was studied. Out of 38 cases with lymph node metastasis, 36 cases (94.7 %) showed a CK-19 positive expression, and 2 case showed a negative expression (5.3 %). All the 122 cases without lymph node metastasis also showed a positive CK 19 expression (100 %) CK19 expression and the lymph node status of the patient does not have a statistically significant relationship as the P value is  $>0.05$  (0.237) (Table 4)

#### Discussion

Present study included 160 total thyroidectomy cases diagnosed as papillary thyroid carcinoma. Out of 160 cases most of the cases were in the age group less than 45 years and with a female preponderance. Primary objective was to study the expression of CK-19 in papillary thyroid carcinoma and to study the association between its expression and the prognostic factors of PTC. The most common variant of papillary thyroid carcinoma obtained in the present study was classic variant, followed by follicular variant. Similar studies done by Sook et al. and many other studies showed common type as the classic variant followed by follicular variant[8]. Regarding the size of the tumour, present studies shows predominantly size more than 1 cm and study conducted by Kaliszewski et al. also showed that the predominant cases in their study were more than 1 cm in size, whereas study conducted by Zeming et al and Sook et al showed that the predominant population in their study were showing a size less than or equal to 1 cm. Present study showed a lymph node metastasis in 23.8 % of cases and the studies conducted by

Kaliszewski et al showed lymph node metastasis in 26.1 % of cases and study conducted by Zeming et al. showed 30.5 % cases with lymph node metastasis which is comparable with our study. The previous studies showed the lymph node metastasis was predominantly seen in the classic variant of PTC similar to present study[9]. Regarding CK-19 expression, 98.75 % of cases showed a CK-19 positivity in the present study and it was comparable with the previous studies. The study conducted by Bose et al. and another study conducted by Gong et al. showed a 100 % positive expression for CK-19 expression and Dina et al. showed 85 % positivity for CK-19 expression in papillary carcinoma thyroid[10].

#### Conclusion

Among PTC cases, classic variant of PTC was the most common. The predominant population was below 45 years of age and there was a female predominance. Majority had a tumour size of more than 1 cm. 23.8 % of cases showed lymph node metastasis. 98.75 % showed a positive CK-19 expression, but there was no association between CK-19 expression and factors like age, gender, tumour size, and lymph node metastasis. 96.3 % of cases showed loss of expression of CD56. Through this study it is concluded that, both CK-19 and CD56 can be used together as markers for PTC including all its variants but CK-19 cannot be used to assess the prognosis of papillary thyroid carcinoma as no association was identified in the study between the expression of CK-19 and prognostic factors.

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