

# Immunohistochemistry on cell blocks a method for pre-operative diagnosis of papillary thyroid carcinoma by FNAC

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## Abstract:

**Background:** Papillary thyroid carcinoma (PTC) is the commonest thyroid cancer. Cases in category-5a of Bethesda system (suspicious for papillary carcinoma) are treated by surgical lobectomy followed by total thyroidectomy if histopathology confirms papillary carcinoma. In order to reduce surgical procedures to one this was conducted.

**Objectives:** evaluation of role of immunohistochemistry in pre-operative diagnosis of papillary thyroid carcinoma on cell blocks.

**Materials and Method:** Cell blocks were taken from cases labelled category-5a for histopathology and immunohistochemistry using three markers (CK-19, Thyro-peroxidase, and BRAF<sup>V600E</sup> mutation).

**Results:** were highly sensitive, and specific. The use of more than markers increases sensitivity of the procedure.

**Conclusion:** immunohistochemical stains on cell blocks is a reliable method for pre-operative diagnosis of papillary thyroid carcinoma.

**Keywords:** Papillary thyroid carcinoma, FNAC, Cell blocks, IHC.

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## Introduction:

Papillary thyroid carcinoma (PTC) is the commonest malignant thyroid tumor constituting 83% of thyroid cancer [1]. Clinical and sonographic suspicious thyroid nodules are managed by fine needle aspiration cytopathology (FNAC) [2, 3]. In order to unify terminology and increases communication between pathologists and clinicians Bethesda system for reporting thyroid cytopathology was introduced [4, 5]. It includes six categories, with approximate percentage of occurrence, risk of malignancy of each category and suggested management, table-1. Category-4a includes cases suspicious of papillary thyroid carcinoma. These cases are treated by lobectomy and followed by total thyroidectomy if histopathology confirm papillary thyroid carcinoma. Two stages operation will have a burden on health and financial state of patients. In order to overcome this problem, the current study was designed for pre-operative confirmation or exclusion of papillary thyroid carcinoma using three immunohistochemical markers (Cytokeratin-19, Thyro-peroxidase and BRAF V600E). They are applied on cell blocks taken from FNAC in order to reduce the number of two stages surgery. Cytokeratin-19 (CK19) is present in most cells of papillary thyroid carcinoma. It has a low molecular weight and present in basal cell and epithelium [6, 7]. Thyro-peroxidase (TPO) is an enzyme essential in thyroid function and iodine metabolism. It is present in normal and benign thyroid tissues but negative in papillary thyroid carcinoma [6, 8]. The common genetic mutation in papillary thyroid carcinoma is (BRAF) gene

mutation which results in substitution of valine to glutamic acid in codon 600 (BRAF<sup>V600E</sup>). This mutation is not seen in benign or other malignant thyroid lesions [9, 10].

**Table-1: Diagnostic categories of Bethesda system for reporting thyroid cytopathology.**

Diagnostic category	Risk of malignancy	Management
1. Non-diagnostic or unsatisfactory	5–10%	Repeat FNA with ultrasound guidance.
2. Benign	0 – 3%	Clinical and sonographic follow-up
3. Atypia of undetermined significance or follicular lesion of undetermined significance.	6 – 18%	Repeat FNA, molecular testing, or lobectomy
4. Follicular neoplasm or suspicious for a follicular neoplasm.	10 – 40%	Molecular testing, or lobectomy
5. Suspicion for malignancy.	45 – 60%	Near-total thyroidectomy or lobectomy
6. Malignant.	94 – 96%	Near-total thyroidectomy or lobectomy

## Materials and methods:

Ninety-two patients with clinical, sonographic suspicious thyroid nodule<sub>(s)</sub> underwent FNAC smears and cell blocks were taken. All ninety-two cases were categorized as suspicious for papillary thyroid carcinoma (category-5a). Patients attending Al-Yarmouk teaching hospital/department of surgery in the period from March 2015 to January 2018. They were 64 females and 28 males and their ages range from 14 to 68 years. Patients after

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cytopathologic diagnosis were treated by surgery in form of lobectomy or thyroidectomy.

**Methods**

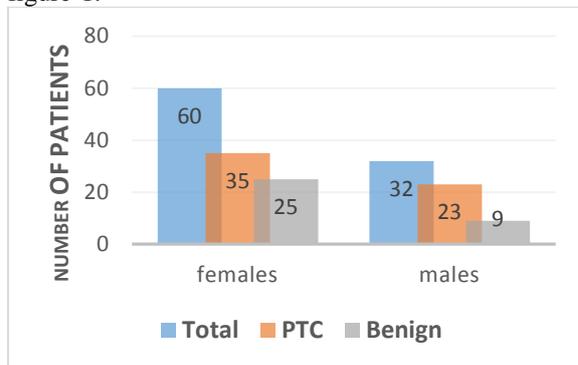
Fine needle aspiration (FNAC) was performed under ultrasound, smears taken for cytopathology and remaining materials for cell blocks. Immunohistochemistry for CK-19, TPO, and BRAF<sup>V600E</sup> were performed. After diagnosis by FNAC as category-5a the mass was removed by lobectomy or thyroidectomy followed by histopathology. Results of histopathology were taken as gold standard method for diagnosis. The work was achieved in department of pathology/Al-Mustansiriyah college of medicine. Study was approved by College Ethical Committee and signed consents were taken from all participants.

**Statistical analysis:**

Microsoft 2010 and IBM 24 version soft wares employed in the statistical analysis. Results of numerical data as standard error and mean. The *t-test* was used for comparison between two groups when data were normally distributed. Data that were not normally distributed were assessed by Mann-Whitney U test. Data with *p-value* <0.05 were considered statistically significant.

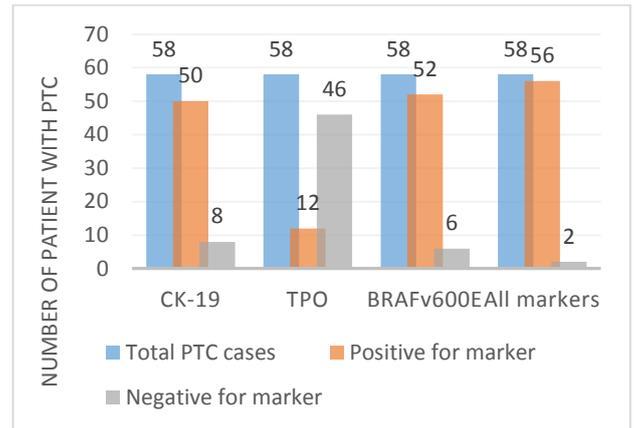
**Results:**

**Clinical findings:** Total number of patients was 92, 60 were females and 32 were males. Thirty-five of females and 23 of males have papillary thyroid carcinoma by histopathology. Twenty-five of females and 9 of males have benign thyroid lesions, figure-1.



**Figure-1: Clinical and histopathologic findings.**

Immunohistochemical findings on cell blocks: Cytokeratin-19 (CK-19) was positive in 50 cases of papillary thyroid carcinoma (86.21%) and negative in 8 (13.79%). CK-19 is negative in all benign thyroid lesions. Thyro-peroxidase (TPO) was negative 46 cases of PTC (79.31%) and positive in 12 (20.69%). PTO was positive in all benign cases. BRAF<sup>V600E</sup> mutation was positive in 52 (89.66%), negative in 6 (10.34%) cases of PTC, and negative in all benign thyroid conditions. Employing all three markers together 56 (96.55%) were positive and 2 (3.45%) were negative figure-2.



**Figure-2: Immunohistochemical findings in cases of papillary thyroid carcinoma.**

**Discussion**

The histopathology results of resected specimens were considered as a gold standard method for diagnosis, i.e. cases with papillary carcinoma are considered as papillary carcinoma and benign cases are benign despite the results of IHC. Histopathology on resected specimens from cases of category-5a (suspicious for papillary carcinoma) showed papillary thyroid carcinoma and benign conditions in 63.04% and 36.96% respectively. This coincides with risk of carcinoma in category-5a of Bethesda system, and with other international studies [3, 4, 5]. Present work showed the studied markers were perfectly stained in cell blocks taken from FNAC of thyroid. On IHC, CK-19 was positive in 50/58 (86.21%), negative in 8/58(13.79%) cases of papillary thyroid carcinoma (PTC). CK-19 was negative in all benign thyroid cases (no false positive results seen). The sensitivity and specificity of CK-19 in diagnosis of PTC were 86.21% and 100% respectively. These findings are in agreement with studies by Hemanathan Guhanandam et al and Haiyan Liu et al [6,7]. Thyro-peroxidase (TPO) is present in normal and benign thyroid tissues but negative in most cases of papillary thyroid carcinoma [7, 8]. In current study it was negative in 46/58 (79.31%), positive in 12/58 (20.69%) of PTC cases diagnosed by histopathology. The TPO was positive in all benign cases (no false positive results). The sensitivity and specificity of TPO were 79.31%, and 100% respectively. The IHC findings of TPO were also in agreement with other international studies by Liu Z et al [12], and Lihua Huang [13]. BRAF<sup>V600E</sup> mutation by IHC was positive in 52 (89.66%), negative in 6 (10.34%) of cases of thyroid papillary carcinoma, and it is also negative in all benign thyroid conditions. The sensitivity and specificity of BRAF<sup>V600E</sup> in papillary thyroid carcinoma approaching 89.66%, and 100% respectively. There is no positive mutation of BRAF<sup>V600E</sup> in all benign conditions (there is no false positive results). Results of BRAF<sup>V600E</sup> mutation in the current study is consistent with other internal studies by Najla Fakhruddin et al [14], and Efsio

Puxeddu et al [15]. The sensitivity and specificity of the three markers together by IHC (CK-19, TPO, and BRAF<sup>v600E</sup>) were 96.55%, and 100% respectively.

#### Conclusion:

Any thyroid lesion positive for CK-19, BRAF<sup>v600E</sup>, and/or negative for TPO is considered as a thyroid papillary carcinoma and the use of more than one marker increases the sensitivity of the procedure.

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