Diagnostic Value of CK19, HBME-1 and TROP2 Biomarkers in Identification of Different Types of Thyroid Follicular Neoplasms

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Abstract- Thyroid malignancies are found in 7% to 15% of all thyroid nodules. Immunohistochemical markers, including CK19, HBME-1and TROP2, have shown an effective role in identifying these malignancies. Hence, due to the lack of appropriate diagnostic tests for the identification of thyroid neoplasms, in this study, we aimed to determine the diagnostic value of these biomarkers in the identification of different types of follicular thyroid neoplasms. In this cross-sectional study, paraffin-embedded tissue blocks from the surgical resection of patients with thyroid nodules, referring to Imam Reza and Razavi Hospitals of Mashhad in 2017, were studied. Sensitivity, specificity, and positive and negative predictive values of these biomarkers for the identification of different types of follicular thyroid neoplasms were also studied. 129 patients with a mean age of 44.65±12.59 years participated in this study, of whom 101 (78.29%) were women. The most common type of follicular thyroid neoplasm was papillary carcinoma (60.47%). The highest sensitivity (94.87%) and positive predictive value (68.51%) in the detection of follicular neoplasms was observed by CK19 in papillary carcinoma. The sensitivity and positive predictive value of TROP2 in the detection of papillary neoplasms was 93.58% and 75.25%, respectively. In addition, HBME-1 had the highest specificity (72.54 %) and positive predictive value (81.57%) in identifying this neoplasm. The results of this study showed that CK19, HBME-1, and TROP2 had high diagnostic value in the detection of papillary thyroid neoplasms. Although these biomarkers had low diagnostic value in identifying follicular adenoma and carcinoma, given the high negative predictive value, they can be considered as powerful markers in identifying negative cases.

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Introduction

The thyroid is an endocrine gland that, like the pituitary gland, plays a vital role in human health and regulates the metabolism in the body (1). Thyroid malignancies are very common, and they account for about 2.5 percent of all malignancies, comprising 95 percent of endocrine system tumors. Epidemiologically, the prevalence of thyroid cancer is higher in women, making it the sixth most common cancer in women (2,3). Moreover, the frequency, as well as the risk of thyroid cancer occurrence, increases with age. Papillary and follicular carcinomas have the highest incidence among other thyroid cancers (4). Thyroid follicular neoplasms are divided into two types of benign and malignant, and each group contains different subtypes (5,6). Benign thyroid follicular neoplasms include follicular adenoma and thyroid cyst. Thyroid malignant follicular neoplasms include papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), anaplastic thyroid carcinoma (ATC), and poorly differentiated thyroid carcinoma (PDTC). The most frequent type of thyroid malignancy is PTC, with 60-70%. Also, there are different varieties of PTC with different prognoses, including Classic, Follicular, Hobnail, Columnar, Tall cell, Cribriform-morular, Solid, and Sclerosing (7).

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Diagnostic methods used in thyroid cancer include physical examinations, thyroid tests, and fine-needle aspiration (FNA), of which the FNA is the most reliable method (8). The sensitivity of FNA for the detection of malignancy in thyroid nodules is ≥90%, but its specificity is 50-65% due to false-positive records, and as a result, unnecessary surgical excision is sometimes performed (9). Given the above, complementary techniques are needed to improve the screening capability of these cytological classifications. Molecular methods that have been introduced for this purpose are very costly, while immunohistochemical biomarkers, as low-cost techniques, may enhance the diagnostic value of FNA.

Findings have shown that immunohistochemical biomarkers such as trophoblast cell-surface antigen 2 (TROP2), Hector Battifora mesothelial-1 (HBME-1), and Cytokeratin19 (CK19) may have diagnostic value in the detection of different malignancies (10,11). Also, it has been shown that these biomarkers may be useful in the identification of different thyroid follicular neoplasm and also the classification of tumors into high and low-risk malignancies (12,13). TROP2 is a human trophoblastic marker that is a cell surface glycoprotein that is encoded by a gene from the TACTD2 gene family located on P32 of chromosome 1. The protein of this gene is typically overexpressed in human carcinomas. HBME-1 is a monoclonal antibody for an antigen of unknown microvillus on mesothelial and epithelial cells. Recent studies have shown an association between the expression of HBME-1 and papillary carcinoma. Similarly, findings have shown that CK19 is highly expressed in patients with PTC (14). To improve the accuracy of the FNA method, the present study aimed to evaluate the diagnostic value of these biomarkers in the identification of different thyroid follicular neoplasm.

Materials and Methods

Sample collection and inclusion criteria

Paraffin-embedded tissue samples of the patients with thyroid nodules that were resected surgically at Imam Reza and Razavi Hospitals of Mashhad in 2017 were studied. The thyroid nodules of the resected tissues were first evaluated histologically and confirmed by an expert pathologist. Inclusion criteria were patients with confirmed different subtypes of FTC including follicular adenoma, PTC, FTC, ATC, and PDTC. Hence, patients with confirmed medullary thyroid carcinoma, mixed medullar and follicular carcinoma, primary non-epithelial and secondary thyroid tumors, and also non-identified thyroid nodules were excluded from further evaluation.

Tissue evaluation

Paraffin-embedded tissue samples were evaluated for immunohistochemical biomarkers including TROP2, HBME-1, and CK19. For this purpose, the samples were first cut into 1 µm sections and then placed on thepoly-L-lysine slide. Positive and negative controls for the CK19 were breast tissue samples (ductal and secretory cells) and hepatocytes, respectively. Similarly, mesothelioma and placenta were used as positive controls for HBME-1 and TROP2, respectively. The negative controls for these biomarkers were normal thyroid tissues. After deparaffinization, the tissue samples were hydrated with alcohol and xylene each step for 5 min. Antigen recovery was performed with EDTA-Tris at 98° C for 20 min. After cooling the temperature to 25° C, the tissue blocks were washed with Tris buffer solution (TBS), and then a 3% H_2O_2 solution was added to stop the reaction. Afterward, the block sections were washed with Tris buffer and were then treated with the ready to use primary antibodies according to the instruction. The tissue sections were then washed with a post-primary block for 20 min, washed with TBS, and incubated with Novolink polymer for an additional 20 min. Then, the sections were washed with TBS, incubated with diaminobenzidine-based peroxidase substrate for xx min, washed with water, and treated with hematoxylin for 3 min. After washing again with water, the sections were dehydrated and placed on a slide for microscopy evaluation. The results were considered positive if at least 10% of the cells were stained. Otherwise, the result was negative for the studied biomarker (36). A positive result for CK19 was membranous staining with or without cytoplasmic staining, but for HBME-1 and TROP2, only membranous staining was considered a positive result.

After the described procedure, the frequency of positive samples for the described biomarkers was studied and compared to normal tissues. Finally, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each marker in the identification of different types of thyroid neoplasms were evaluated.

Statistical analyses

Regarding sample size, because the frequency of thyroid follicular neoplasm is very low among the

general population, all identified patients with different types of thyroid carcinomas who referred to Imam Reza and Razavi hospital of Mashhad in 2017 were included in this study.

Statistical analyses were performed with SPSS statistical software package (version 22). Continuous variables are expressed as mean±standard deviations, and categorical variables are distributed as numbers and percentages. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the markers mentioned in the identification of each of the different types of thyroid neoplasm were determined and reported as a percent.

Results

During the year 2017, 135 patients with thyroid neoplasm who underwent resection surgery were included, of whom 129 patients fully met the inclusion criteria and involved in the study. The mean age of participants was 44.65±12.59 (age range 18-81). Of the included patients, 101 (78.29%) were female, and 28 (21.71%) were male. The results of this study showed that the frequency of PTC was greater than 78 (60.47%) among other types of thyroid carcinoma, and follicular adenomas were the second greatest type of thyroid neoplasms with a frequency of17.83%. Hurthle cell carcinoma is a subtype of follicular carcinoma but with different biological properties was the third carcinoma with the greatest frequency (9.3%). Other types of thyroid carcinomas include noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and follicular carcinoma with the frequency of 6.98% and 5.43%, respectively. Also, the results showed that the highest frequency of papillary carcinoma variants belonged to the classical type (84.61%), and the frequency of follicular and Tall cell variants were 11.54% and 3.85%, respectively.

The findings of the present study showed that the CK19 sensitivity for identification of NIFTP and PTC was 100% and 94.87%, respectively. The sensitivity of this marker for the identification of follicular carcinoma, follicular adenoma, and Hurthle cell carcinoma was 85.71%, 60.87%, and 41.67%, respectively. On the other hand, findings showed that CK19 was more specific to PTC (33.34%). Also, the highest PPV of CK19 was 68.51% for PTC, while the highest NPV of this marker was 95.23% for follicular carcinoma. Table 1 shows the diagnostic potential of CK19 for the identification of different types of thyroid neoplasms.

Table 1. Diagnostic value of the CK19 in the identification of different thyroid carcinomas

CK19	PTC (%)	Follicular carcinoma (%)	Follicular adenoma (%)	Hurthle cell carcinoma (%)	NIFTP (%)
Sensitivity	94.87	85.71	60.87	41.67	100
Specificity	33.34	16.39	11.33	11.96	22.12
PPV	68.51	5.5	12.96	4.6	8
NPV	80.95	95.23	57.14	66.67	100

PTC: papillary thyroid carcinoma, NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features, PPV: positive predictive value, NPV: negative predictive value

Also, the results showed that TROP2 is a helpful biomarker for the identification of different thyroid follicular neoplasms. Findings showed that the sensitivity and specificity of this marker for PTC was 93.58% and 52.94%, respectively. Also, the PPV and NPV of TROP2 for PTC were 75.25% and 84.37%, respectively. TROP2 had low PPV for carcinomas and adenomas (2.06% and 8.24%, respectively). The identification of Hurthle cell carcinoma by TROP2 was successful in 50% of the cases, but the PPV was 6.18%. The sensitivity, specificity, and diagnostic value of TROP2 for the identification of other thyroid neoplasms are summarized in Table 2.

HBME-1, another diagnostic marker, showed 79.48% sensitivity and 72.54% specificity for the identification of PTC. Also, the PPV and NPV of this marker for PTC were 81.57% and 69.81%, respectively. It also has a high NPV for follicular thyroid neoplasm (90.56%) and Hurthle cell carcinoma (77.35%), indicating that HBME-1 may be a helpful marker for negative cases of follicular thyroid neoplasm and Hurthle cell carcinoma. The diagnostic value of HBME-1 for the detection of other types of thyroid neoplasm is summarized in Table 3.

Evaluation of the diagnostic value of CK19, TROP2, and HBME-1 in the detection of papillary carcinoma variants revealed that CK19 had the highest sensitivity (95.45%) for detection of classical variant and although the sensitivity of this biomarker was 100% for follicular and Tall cell variants, due to the small sample size these results were not valuable. PPV of CK19 for classical, follicular, and Tall cell variants were 85.15%, 1.69%,

and 4.05%, respectively. Also, the sensitivity of TROP2 for classical, follicular, and Tall cell variants was 93.94%, 88.89%, and 100%, respectively, and the specificity of this marker was 8.03%, 5.79%, and 6.67% for the described variants, respectively. PPV of TROP2 for classical, follicular, and Tall cell variants were 84.93%, 10.95%, and 4.1%, respectively. In addition, HBME-1 had 83.34% sensitivity for classical variant, 77.78% for follicular, and 100% for Tall cell. The specificity of HBME-1 for these variants was 41.66%, 20.28%, and 21.33%, respectively, and the PPV of HBME-1 for classical, follicular, and Tall cell variants were 88.70%, 11.29%, and 4.83%, respectively. The results also showed that simultaneous use of CK19 and TROP2 had a sensitivity of 100%, 98.72%, 66.66%, 73.91%, and 85.71% for NIFTP, papillary carcinoma, Hertel cell carcinoma, follicular adenoma, and follicular carcinoma, respectively. The specificity of these biomarkers in papillary carcinoma, follicular carcinoma and adenoma, Hertel cell carcinoma, and NIFTP were 17.64%, 4%, 1.88%, 2.5%, and 5%, respectively. Also, it was shown that the concurrent use of CK19 and HBME-1 was more sensitive for NIFTP and papillary carcinoma (100% and 98.71%, respectively). Moreover, the diagnostic value of concomitant use of TROP2 and HBME-1 was evaluated, and the highest sensitivity belonged to NIFTP and papillary carcinoma (100% and 98.71%, respectively) (Table 4).

Table 2. Diagnostic potential of the TROP2 in the identification of thyroid carcinomas

TROP2	PTC (%)	Follicular carcinoma (%)	Follicular adenoma (%)	Hurthle cell carcinoma (%)	NIFTP (%)
Sensitivity	93.58	28.57	34.87	50	88.89
Specificity	52.94	22.13	16.03	22.23	25.83
PPV	75.25	2.06	8.24	6.18	8.24
NPV	84.37	84.37	53.12	81.25	96.87

PTC: papillary thyroid carcinoma, NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features, PPV: positive predictive value, NPV: negative predictive value

Table 3. Diagnostic value of HBME-1 biomarker in the identification of thyroid carcinomas

HBME-1	PTC (%)	Follicular carcinoma (%)	Follicular adenoma (%)	Hurthle cell carcinoma (%)	NIFTP (%)
Sensitivity	79.48	28.57	21.74	0	77.78
Specificity	72.54	39.34	33.01	35.04	42.5
PPV	81.57	2.63	6.57	0	9.21
NPV	69.81	90.56	66.03	77.35	96.22

PTC: papillary thyroid carcinoma, NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features, PPV: positive predictive value, NPV: negative predictive value

Table 4. The results of simultaneous use of CK19, TROP2, and HBME-1 biomarker in the identification of thyroid follicular neoplasms

Type of neoplasm	TROP2 and CK19		CK19and HBME-1		TROP2 and HBME-1	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Papillary carcinoma	98.72	17.64	98.71	23.52	98.71	39.21
Follicular carcinoma	85.71	4	85.71	6.55	42.85	15.57
Follicular adenoma	73.91	1.88	60.86	3.66	34.78	5.66
Hurthle cell carcinoma	66.66	2.5	41.66	4.27	50	7.69
NIFTP	100	5	100	7.5	100	10.83

The values are described as the percent.
NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features

Discussion

There have been numerous studies on the role of HBME-1, CK19, and TROP2 biomarkers in the identification of thyroid neoplasms. Although the results are conflicting, the results of different studies have shown that the mean sensitivity and specificity of CK19 for detection of papillary carcinoma were 85.4% and 82.29%, respectively. Moreover, the mean sensitivity and specificity of HBME-1 was 78.3% and 85.4%, respectively (15-17). According to Simms et al., TROP2 had 95.5% sensitivity and 89% specificity in identifying papillary carcinoma (9). Findings have also shown that HBME-1 is one of the most reliable markers for the identification of thyroid pathology (18). Overall, this marker had a lower diagnostic value for all types of neoplasms than the other two markers.

Although studies have shown that CK19 is negative

in most of the normal thyroid tissue, including follicular adenoma (19,20), in the present study, CK19 was positive in 60.87% of follicular adenoma cases. This marker also had the highest NPV (95.23%) in the diagnosis of FTC, followed by HBME-1 and TROP2 with NPV of 90.56% and 84.37%, respectively. Also, studies have shown that TROP2 is not usually FTC-positive, and our finding was consistent with these reports, and only 28.57% of the cases were positive for FTC using TROP2 and HBME-1. According to our findings, TROP2 was the second most sensitive marker (93.58%) with the highest NPV (84.37%) in detecting PTC. But the highest specificity (72.54%) with the highest PPV (81.57%) in the diagnosis of PTC belonged to HBME-1. On the other hand, the results of a study by Chen *et al.*, showed that the expression of HBME-1 was not significantly associated with age, sex, and the size of the papillary thyroid nodule. Similar to our findings, their results also showed that HBME-1 has the highest sensitivity and specificity for the diagnosis of PTC (21). Also, inconsistent with our findings, Zhu *et al.*, found that the expression of CK19 and HBME-1 was significantly higher in patients with PTC than the healthy controls. They also reported that the expression of CK19 was significantly higher in patients with papillary carcinoma than in those with follicular carcinoma (22). In addition, evaluation of the diagnostic value of TROP2, HBME-1 and CK19 in papillary thyroid cancers showed that 73.8%, 83.3% and 50% of all PTC cases were positive for HBME-1, CK19 and TROP2, respectively (16). In our study, 95.94%, 94.59% and 79.73% of all PTC cases were positive for CK19, TROP2 and HBME-1, respectively.

Similarly, Nga *et al.*, in 2008 showed that the sensitivity of CK19 and HBME-1 in patients with papillary carcinoma was 100% and their specificity was 84% and 92%, respectively. PPV of CK19 and HBME-1 were also reported to be 82% and 90%, respectively (22). In our study, sensitivity, specificity and PPV of CK19 were 94.87%, 33.34% and 68.51%, respectively, and these values for HBME-1 were 79.48%, 72.54% and 81.57%, respectively. Also, Liu et al in 2015 showed that the expression levels of CK19, TROP2 and HBME-1 were significantly higher in malignant neoplasms than in benign neoplasms. They also reported that among these biomarkers, CK19 had the highest sensitivity (96.30%) and the lowest specificity (40%) to detect PTC, which was consistent with the results of our study (14). In the present study, the highest diagnostic value in differentiating all types of neoplasms belonged to HBME-1. The results also showed that concurrent use of these markers could increase the sensitivity in diagnosis of PTC.

Findings of this study indicate that CK19, HBME-1, and TROP2 have high diagnostic value in the detection of thyroid neoplasms of papillary origin. Although these markers have low diagnostic value in the diagnosis of follicular carcinoma, but given the high negative predictive value, they may have a high potential for detecting negative cases.

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