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Scoring System and Diagnosis of Papillary Thyroid Carcinoma Using Human Bone Marrow Endothelium Marker-1, Cytokeratin 19, and Galectin-3

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Abstract

Background: The increasing incidence of papillary thyroid carcinoma (PTC) and the inadequacy of routine histologic examination in its diagnosis necessitate the application of ancillary studies like immunohistochemistry. This research aimed to investigate the scoring system and diagnosis of PTC with cytokeratin 19 (CK19), human bone marrow endothelium marker-1 (HBME-1), and galectin-3.

Methods: This experimental laboratory study was performed at Babol University of Medical Sciences, Mazandaran, Iran from April 2017 to March 2019. Neoplastic and nonneoplastic tissue samples of 100 cases with a diagnosis of PTC were selected by convenience sampling. CK19, HBME-1, and galectin-3 immunohistochemistry markers were used on tissue samples. Analysis was performed using the t test and the chi-square test, as well as the receiver operator characteristic (ROC) curve (significance level $P < 0.05$).

Results: The CK19 staining was observed in all 100 (100%) non-neoplastic tissues, but HBME-1 and galectin-3 were positive in 36 (36%) and 14 (14%) of non-neoplastic tissues, respectively. The intensity scores of all the markers and their total had significantly different means in PTC and non-neoplastic tissues ($P < 0.001$). A significant difference was observed between the total score of each marker and the total score of their combination ($P < 0.001$). The combination of all 3 markers with an 11.5 0 cut-off for the total score showed the most sensitive (0.99) and specific (1.00) results.

Conclusion: Interpreting CK19, HBME-1, and galectin-3 with the aid of the proposed scoring system was fruitful. HBME-1 and galectin-3 can be used individually or in combination for the diagnosis of PTC.

Keywords: Papillary Thyroid Carcinoma, Non-neoplastic, Keratin 19, Galectin-3

Conflicts of Interest: *None declared*
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Introduction

There are numerous published reports on the rise in the incidence of thyroid carcinoma (1). The epidemiologic studies in the United States revealed a 3.6% increase in the prevalence of this type of cancer (2). In addition, research studies showed that the incidence rate (per year) of thyroid cancer in Iran was 2.2 per 100,000 persons between 2004 and 2010. The most common type of cancer in Iran in terms of histology was PTC, with an annual rate of 0.29 (3). Various studies in Iran reported that the incidence of thyroid cancer increased from 2003 to 2009 (4). Another study in Iran showed that 1545 patients were diagnosed with thyroid cancer from 2011 to 2015, from which 3% was related to

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↑What is "already known" in this topic:
Immunohistochemical markers are highly sensitive and specific and improve diagnostic accuracy. The accuracy of cytokeratin 19 (CK19), human bone marrow endothelium marker-1 (HBME-1), and galectin-3, separately or in combination, is important in the diagnosis of benign and malignant thyroid lesions before and after surgery.

→What this article adds:
The use of HBME-1 and galectin-3 separately can be used to diagnose PTC. However, their combination for PTC diagnosis is more sensitive and specific.

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death events (5). This increase in the number of cases is due to the development and extensive implementation of imaging studies (6, 7). Among all types of thyroid cancer, the most common type is papillary thyroid carcinoma (PTC), accounting for more than 80% of cases (8). A great number of cases are subclinical in which PTC sizes is <1 cm. Some studies have shown that the observed increase of thyroid carcinoma is a result of detecting small-sized PTCs (1, 3). It has been proposed that the increased number of PTC cases can be due to correct diagnosis, increased diagnostic scrutiny with advanced techniques, and the true nature of the cancer (1). The process of diagnosis ends with histopathology of tissue specimens. The small-sized thyroid carcinomas are almost always papillary microcarcinoma and they could be found in specimens collected for other conditions like multinodular goiter (9). The main tool in surgical pathology is evaluation of haemotoxylin and eosin (H&E) sections. With regard to PTC, some diagnostic conflicts rose many years ago and numerous attempts have been made to solve the issue. The list of problems is long. For example, both poor fixation and extensive calcification, which require decalcification, can cause artifactual nuclear clearing. Chronic lymphocytic thyroiditis can result in reactive nuclear change to the degree which cannot be distinguished from nuclear atypia observed in PTC. Nuclear chromatin clearing, groove, pseudo-inclusion, and membrane thickening can be found in Hashimoto thyroiditis. Papillary formation is another source of diagnostic difficulty. It can be found in many non-neoplastic conditions, including Grave's disease, Hashimoto thyroiditis, and multinodular goiter. In most cases, the absence of typical PTC nuclear features helps in distinguishing it from malignancy, but some cases with oncocytic change can have nuclear groove or pseudo-inclusion like nuclear holes. Reactive changes after fine needle aspiration can also cause errors (10). All the mentioned issues imply that conventional H&E sections are not enough, and for this very purpose, immunohistochemistry (IHC) has been tried in many studies (11-13). Nechifor-Boilă et al reported that CD56 (81.8%), HBME-1 (63.6% sensitivity), cytokeratin-19 (45.6%), and galectin-3 (100%) were the most sensitive markers for PTC and thyroid tumors detection (14). Ramkumar et al showed that HBME-1 and galectin-3 expressions and BRAF V600E mutation in thyroid neoplasms, individually and combined, are specific ancillary diagnostic techniques for PTC detection (15). Wu et al showed that combinations of HBME-1 with CK-19, galectin-3, or HER-2/neu were the most specific ones (98.3 %) and could improve the specificity of PTC diagnosis (16). A variety of methods, IHC markers, and different panels have been proposed for diagnosing benign and malignant thyroid lesions. Usually, because of the staining observed in non-neoplastic tissue, the exact IHC panel is not obtained from them. Therefore, the use of these methods in combination with each other can make the diagnosis method more accurate. Since each of the markers CK19, HBME-1, and galectin-3 is among the most sensitive markers, the combination of these 3 methods can be very effective in diagnosis of PTC and adjacent non-neoplastic tissue, and this proposes an efficient scoring system for PTC, which can achieve accurate results (14-16). Therefore, evaluation of each of these 3 markers in PTC diagnosis, comparing their staining patterns in PTC and adjacent non-neoplastic tissue, and presenting an efficient scoring system for grading and diagnosis of PTC were considered in this study.

Methods

Sample Preparation

This experimental laboratory study was conducted at Babol University of Medical Sciences, located in Babol, Mazandaran province, Iran (Ethic code: IR.MUBABOL.HRI.REC.139802440). The archives of the pathology departments of all the public hospitals of Babol were searched for cases with diagnosis of PTC from April 2017 to March 2019, and 116 tissue-embedded paraffin blocks were found (Convenience sampling). Sixteen (13.79%) of 116 tissue-embedded paraffin blocks were removed due to the lack of suitable paraffin blocks for H&E staining. The ideal fixation time will depend on the size of the tissue block, and removal of paraffin can cause poor staining of the section (13). They did not have enough natural surrounding tissue. Finally, 100 (86.20 %) samples were considered.

Then, the conventional H&E-stained sections of all the available paraffin blocks were selected and reviewed by 2 pathologists. H&E is a combination of 2 tissue dyes: hematoxylin and eosin. Hematoxylin stains the nucleus of cells as purple, the matrix as eosin blue, and cytoplasm as pink. Other structures in the cell take on a variety of shades, colors, and other combinations of these colors. Thus, a pathologist can easily distinguish between the nucleus and the cytoplasm of the cell, and in addition, it shows the general staining patterns of cell design and distribution, and provides an overview of the structure of the tissue sample (17, 18). The diagnosis of PTC was confirmed in the presence of papillary structures with fibrovascular core lined by follicular cells with characteristic features including enlarged and elongated nuclei with overlapping, irregular contours, groove, pseudo-inclusion, and optically clear chromatin patterns. The surrounding tissue was designated as non-neoplastic if it had follicles containing colloid and was lined by bland follicular cells with small round basally-located nuclei without crowding, overlapping, or PTC type nuclear features. Sixteen cases were excluded due to the lack of characteristic features of PTC, presence of suspicious elements in the non-neoplastic tissue, and inadequacy or poor quality of tissue. Immunohistochemistry was performed for HBME-1 (Diagnostic BioSystems,), galectin-3 (Diagnostic BioSystems, California, USA) clone DBM15.67, and CK19 (diagnostic BioSystems) clone A53-B/A2.26 (19).

Markers Consideration

The prepared slides were examined by 2 pathologists. Markers were considered positive if at least 1% of the cells showed membranous staining in HBME-1, cytoplasmic staining in CK19, and cytoplasmic and nuclear staining in galectin-3. The difference between PTC and non-neoplastic tissue for each marker was calculated as follows: The

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scores obtained for each marker in terms of intensity and percentages were added together. Then, in each part, PTC markers were deducted from non-neoplastic markers. The percentages of positive cells in both PTC and non-neoplastic tissue were recorded and converted into a five-tier scoring system: <1%, 2%-25%, 26%-50%, 51%-75%, and 76%-100%, which were scored 0 to 4, respectively. Traditionally, the intensity and volume of staining of these markers were visually measured and scored by pathologists in categories of no, weak, intermediate, and strong. Intensity of staining was also recorded in neoplastic and non-neoplastic tissues as no, weak, intermediate, and strong, which were scored from 0 to 3, respectively. The total score were numbers that ranged from 0 to 7 (20).

Data Analysis

In the first step, if the staining was observed in more than 1% of the cells, it was considered positive with no regards to intensity of staining. Then, the scores were compared. Receiver operator characteristic (ROC) curve for discriminating the ability of the total score of each IHC marker and the combination of 2 markers and the total score of all 3 markers (sensitivity versus (1-specificity)) was used.

Data were entered and analyzed in SPSS V. 22 software program (SPSS Inc). A t test for comparing the intensity score of each marker between PTC and non-neoplastic tissues and the chi-square test for assessing the relation of groups with markers were performed (significance level, $P < 0.05$).

Results

The results showed that in the PTC group**,** all (n = 100) of the PTC tissues were positive for HBME-1 (n = 99; 99%), CK19 (n = 100; 100%), and galectin-3 (n = 100; 100%), except 1 (1%) that was negative for HBME-1. The CK19 staining was observed in all (n = 100; 100%) non-neoplastic tissues, which made this a useless marker in diagnosis of PTC, if it was reported either positive (n = 100; 100%) or negative (n = 0; 0%). In the non-neoplastic group, HBME-1 and galectin-3 were negative to lesser extents of 36% (n = 36) and 14% (n = 14), respectively, which were significantly different in the case and control groups ($P < 0.001$) (Table 1).

Figure 1 shows a positive staining in both carcinoma and

Table 1. Immunohistochemistry results of marker expression

Marker Expression	PTC Group N (%)	Non-neoplastic N (%)	*P* Value
HBME-1 (negative)	1(1)	36 (36)	<0.001
HBME-1 (positive)	99 (99)	64 (64)	
Galectin-3 (negative)	0 (0)	14 (14)	<0.001
Galectin-3 (positive)	100 (100)	86 (86)	
CK19 (negative)	0 (0)	0 (0)	None*
CK19 (positive)	100 (100)	100 (100)	

*N, number; none, PTC group n (%) and non-neoplastic n (%) were zero, thus, the chi-square test was not performed.

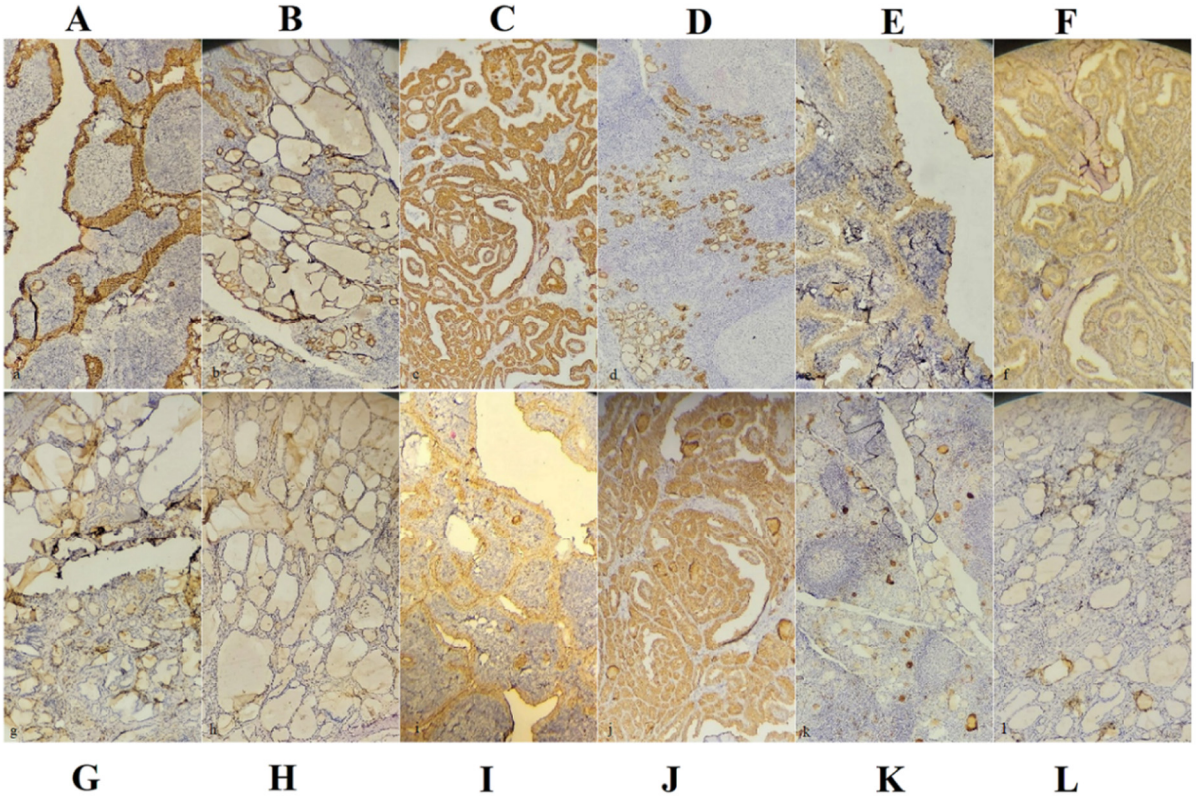

Figure 1. Staining of carcinoma and non-neoplastic tissues, CK19 (A and C, carcinoma; B and D, none-neoplastic), galectin 3 (E and F, carcinoma; G and H, non-neoplastic), HBME-1 (I and J, carcinoma; K and L; non-neoplastic), (X100 fields).

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non-neoplastic tissues in all the 3 markers. A and C parts show carcinoma, and a strong CK19 staining is observed in PTC cells. B and D parts show none-neoplastic cells with a weak CK19 staining in PTC cells. E and F parts are also related to carcinoma cells, and weak to intermediate galectin 3 staining can be seen in this part. Moreover, in G and H sections, the weak staining of galectin 3 is observable in the non-neoplastic tissue. An intermediate to strong HBME-1 staining is observed in I and J sections that are related to carcinoma tissues. K and L are observed in a small number of non-neoplastic cells with a weak staining (Figure 1).

HBME-1 (99%) and galectin-3 (100%) were both sensitive, but not specific markers (HBME-1 [36%] and galectin-3 [14%]) for diagnosis of PTC. PTC was used to determine if the positive (HBME-1 [61%] and galectin-3 [54%]) results that confirmed it were more significant than the negative (HBME-1 [97%] and galectin-3 [100%]) results of staining (Table 2).

The intensity score of all the markers (HBME-1, CK19, and galectin-3) and their total score had significantly different means in PTC and non-neoplastic tissues ($P < 0.001$). Table 3 shows the results of staining intensity for each marker and the total of all the 3 markers.

High percentages of mean of positive cells in non-neoplastic and PTC groups were related to CK19 (61.50% and 94.35%, respectively). Furthermore, there was a significant difference between the means of each group for all IHC markers ($P < 0.001$). Table 4 shows the percentage of positive cells in each group.

At first, the means of intensity score and staining volume were calculated and compared separately for each marker. Then, the means of intensity score and staining volume were calculated and compared for a combination of 2 markers. Finally, the means of intensity score and staining volume were calculated and compared for a combination of all the 3 markers. The mean scores of IHC markers, HBME-1 (5.71 versus 1.42), CK19 (6.63 versus 4.75), and galectin-3 (5.21 versus 1.84) were higher in the PTC group compared with the non-neoplastic group. Furthermore, the mean scores of their combination were higher in the PTC group in comparison with the non-neoplastic group as well—HBME-1 and CK19 (12.34 vs 6.17), HBME-1 and galectin-3 (10.92 vs 3.26), and CK19 and galectin-3 (11.84 vs 6.59). The total score of the 3 markers showed a higher score in the PTC group (17.55) compared with the non-neoplastic group (8.01). There was a significant difference between both the total score of each marker and the total score of their combination ($P < 0.001$) (Table 5).

According to the area under the ROC curve, the discriminating ability of the total score of IHC markers was higher than the score of each of them alone. Therefore, combining the markers and the total score of all the 3 markers were good according to the ROC curve. According to the results, the most sensitive and specific marker was galectin-3 (suggested cut-off, 2.05; sensitivity, 098; 1-specificity, 0.90). The combination of the 2 markers revealed that using all the 3 markers could yield the most sensitive (0.99) and specific (1) result with a cut-off of 11.50 for the total score. Table 6 shows the suggested cut-off for the total score of each marker and their combination, as well as their sensitivity and specificity.

Table 2. Sensitivity, specificity, predictive values, and likely ratio

IHC marker	Sensitivity (CI 95%)	Specificity (CI 95%)	Positive predictive value (CI 95%)	Negative predictive value (CI 95%)	Positive likely ratio (CI 95%)	Negative likely ratio (CI 95%)
HBME-1	99 % (97-100)	36 % (27-45)	61 % (53-68)	97 % (97-100)	1.55 (1.33-1.99)	0.03 (0-0.20)
Galectin-3	100 % (100)	14 % (7-21)	54 % (47-61)	100 % (100)	1.16 (1.07-1.26)	None*

IHC, Immunohistochemistry (IHC); CI, Confidence level; None, All tumor samples were positive (Negative LR= [100-sensivity]/ specificity).

Table 3. Results of the T test for intensity score (intensity of staining)

IHC Marker	Group	N	Mean	SD	SE	T Test for Equality of Means *P* Value
HBME-1	Non-neoplastic	100	0.75	0.642	0.064	<0.001
	PTC	100	2.18	0.609	0.061	
CK19	Non-neoplastic	100	1.87	0.418	0.042	<0.001
	PTC	100	2.70	0.541	0.054	
Galectin-3	Non-neoplastic	100	0.90	0.414	0.041	<0.001
	PTC	100	1.70	0.541	0.054	
HBME-1 and CK19	Non-neoplastic	100	2.62	0.77	0.07	<0.001
	PTC	100	4.88	0.89	0.08	
HBME-1 and Galectin-3	Non-neoplastic	100	1.65	0.82	0.08	<0.001
	PTC	100	3.88	0.79	0.07	
CK19 and Galectin-3	Non-neoplastic	100	2.77	0.56	0.05	<0.001
	PTC	100	4.40	0.66	0.06	
Total of 3 markers	Non-neoplastic	100	3.52	0.91	0.09	<0.001
	PTC	100	6.58	0.95	0.09	

SD, Standard deviation; SE, Standard error.

Table 4. Result of the T test for percentage of positive cells

IHC Marker	Mean of Positive Cells in Non-neoplastic % (SD)	Mean of Positive Cells in PTC % (SD)	T Test for Equality of Means *P* Value
HBME-1	6.15 % (8.43)	78.53 % (22.76)	<0.001
CK19	61.50 % (20.66)	94.35 % (11.64)	<0.001
Galectin-3	12.95 % (11.12)	76.69 % (21.22)	<0.001

SD: Standard deviation

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Table 5. Results of the T test on the total score of each marker and the combined scores

IHC Marker	Group	N	Mean	SD	SE	T Test for Equality of Means *P* Value
HBME-1	Non-neoplastic	100	1.42	1.12	0.11	<0.001
	PTC	100	5.71	1.29	0.12	
CK19	Non-neoplastic	100	4.75	1.14	0.11	<0.001
	PTC	100	6.63	0.78	0.07	
Galectin-3	Non-neoplastic	100	1.84	0.83	0.08	<0.001
	PTC	100	5.21	1.19	0.11	
HBME-1 and CK19	Non-neoplastic	100	6.17	1.70	0.17	<0.001
	PTC	100	12.34	1.65	0.16	
HBME-1 and Galectin-3	Non-neoplastic	100	3.26	1.48	0.14	<0.001
	PTC	100	10.92	1.59	0.15	
CK19 and Galectin-3	Non-neoplastic	100	6.59	1.37	0.13	<0.001
	PTC	100	11.84	1.29	0.12	
Total of three markers	Non-neoplastic	100	8.01	1.93	0.19	<0.001
	PTC	100	17.55	1.79	0.18	

SD, Standard deviation; SE, Standard error.

Table 6. Results of ROC analysis for the total score of each marker and their combination

Test Result Variable	Area Under the ROC Curve	SE[a]	P Value	Asymptotic 95% CI[b]		Suggested Cut-off	Sensitivity	1-Specificity
				Lower Bound	Upper Bound			
HBME-1	0.98	0.01	0.001	0.96	1.00	2.50	0.97	0.86
CK19	0.928	0.02	0.001	0.88	0.96	5.50	0.95	0.72
Galectin-3	0.98	0.00	0.001	0.96	0.99	2.50	0.98	0.90
HBME-1 and CK19	0.98	0.01	0.001	0.96	1.00	8.50	0.97	0.92
HBME-1 and Galectin3	1.00	0.00	0.001	0.99	1.00	7.00	0.98	1
CK19 and Galectin-3	0.99	0.00	0.001	0.99	1.00	8.50	0.99	0.96
Total	0.99	0.00	0.001	0.99	1.00	11.50	0.99	1

[a]SE, standard error; [b]CI, confidence level. Values should only be reported based on specificity, thus, values were deducted from 1; 1-specificity, which is the rate of false positives among all cases that should be negative (false positive + true negative).

Table 7. The Descriptive Statistics for Calculation of the Difference Between PTC and Non-neoplastic Tissue Scores (Total Intensity and Percentage) in Each Marker and the Maximum Differences Between Scores

IHC Marker	N	Minimum	Maximum	Mean	SD
HBME-1	100	0.00	7.00	4.29	1.52
CK19	100	0.00	5.00	1.88	0.99
Galectin-3	100	0.00	6.00	3.37	1.27

N: Number, Minimum: minimum score for each marker, Maximum: maximum score for each marker, SD: Standard deviation

Table 8. Area under the curve for the difference between scores in each marker

Test Result Variable	Area Under the ROC Curve	SE[a]	P Value	Asymptotic 95% CI[b]		Suggested Cut-off	Sensitivity	1-Specificity
				Lower Bound	Upper Bound			
HBME-1	1	0	0.001	1	1	4.50	1.00	1.00
CK19	1	0	0.001	1	1	1.50	1.00	1.00
Galectin-3	1	0	0.001	1	1	3.50	1.00	1.00

[a]SE, standard error; [b]CI, confidence level.

The difference between PTC and non-neoplastic tissue scores (total intensity and percentage) was calculated for each marker. The highest score was observed in HBME-1 (mean ± SD: 4.29 ± 1.52) and the lowest score was observed in CK19 (mean ± SD: 1.88 ± 0.99) (Table 7).

The area under the ROC curve was 1 in all the markers, which showed perfect discriminatory ability. The suggested cut-off for the difference between the total score was highest for HBME-1 (4.50) and the lowest for CK19 (1.50) (Table 8).

Discussion

Most of the published studies have not proposed a proper cut-off for their scoring system to distinguish PTC from benign tissues. However, some advanced studies have provided scores. Most studies have used only intensity or percentage of positive cells in their scoring model, but a few of them have utilized both (21-25). In this study, the results of both positive/negative style and a scoring system with application of volume and intensity factors were used. The next step is histopathologic examination of tumors. In this step under and over diagnosis can alter the results of statistical analysis. The major issue in histopathologic examination of tumor is identification of small-sized tumors with manifestations of papillary carcinoma. Numerous efforts have been made to conquer this obstacle. The main tool, which is the focus of many studies, is immunohistochemistry. Thus, many issues arise in this process, including selection of an antibody panel and interpreting the findings. There is a long list of IHC markers that have been tried by pathologists for diagnosis of PTC. Many studies interpreted the markers merely as positive or negative (26-28). Using the 3 markers of CK19, galectin-3, and HBME-1 in the positive/negative manner resulted in observation of significant

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differences between PTC and non-neoplastic tissues in galectin-3 and HBME-1, but CK19 did not show any significant difference. Both HBME-1 and galectin-3 had low specificity in spite of high sensitivity. CK19 has been used in many studies (29-31). This marker stains epithelial cells in many organs and weak staining in benign thyroid tissue has been observed in many studies (11, 29, 31). The incidence of PTC is increasing worldwide. This is partially due to improvement of early detection by imaging modalities. In this study, HBME-1 and galectin-3 were positive in the non-neoplastic group. Moreover, positive staining was observed in both tumoral and non-tumoral tissues in all the markers. HBME-1 and galectin-3 are not specific markers for diagnosis of PTC. There were significant differences between the scores of all the markers in the PTC and non-neoplastic tissue. According to the results, the most sensitive and specific marker was galectin-3 with a suggested cut-off of 2.5. Cheung et al reported focal positivity in benign thyroid tissues, but diffuse staining was a characteristic of classic PTC in their study (32). Similar to the present study, Nechifor-Boilă et al examined HBME-1, galectin-3, and CK19 markers and stated that these markers can be used alone or in panels in identification of PTC. The sensitivity of HBME-1 was 63.6%, and CK19 and galectin-3 had the lowest sensitivity percentages (45.6% each). In this study, the most sensitive marker was galectin-3 (98%), followed by HBME-1 (97%) and CK19 (95%) (14). Furthermore, the results showed that in the PTC group**,** all (n = 100) of the PTC tissues were positive for galectin-3 (100%). Galectin-3 is a beta-galactosyl-binding lectin that is commonly expressed in macrophages, mast cells, and Langerhans cells, and is present in various malignant cells, including thyroid cells. It has been suggested that galectin-3 may also play a role in thyroid malignancy and is strongly expressed in cases of PTC (33, 34). The sensitivity of all the markers in this study was greater than that of the study by Nechifor-Boilă et al. The panel consisting of CK19 and galectin-3 had the highest sensitivity (90.9%). In the current study, this sensitivity was higher (99%) in CK19 and galectin-3 composite panel. Therefore, new panels of antibodies could be used consisting of CK19 and galactin-3 or HBME-1, which are highly sensitive to PTC (14). Achieving similar results to this study, Liu et al reported that it is important to have differential diagnoses of PTC and non-malignant nodules. In their study, tissue samples were obtained from 257 patients with PTC and 149 patients with non-malignant thyroid samples, and immunohistochemical staining was performed for CK-19 and HBME-1. The expressions of HBME-1 and CK-19 for the PTC group were 96.3% and 85.3%, respectively, and for the group of non-malignant thyroid lesions, these expressions were 40.4% and 37.2%, respectively. The results of the study by Liu et al showed that HBME-1 (99%) and CK19 (100%) were expressed in the PTC group. In addition, obtaining similar results, Liu et al showed that the expressions of CK-19 and HBME-1 in PTCs were much higher than benign thyroid lesions, and combining the positive expressions of CK-19 and HBME-1 can improve the detection characteristics of PTC (22). Murtezaoglu et al observed 100% positivity in normal thyroid tissue and 95.5% in the classic variant of PTC, which made this marker sensitive but not specific. They did not find any degree of staining in normal tissue. Their findings revealed 100% specificity and 73.8% sensitivity for this marker. In the current study, sensitivity was 99%, but specificity was very low, about 36%. A scoring model was used for the purpose of improving specificity. The suggested cut-off was 2.5 with 97% sensitivity and 86% specificity. These findings implied that this marker can be used individually without the need of other markers. The positive staining observed in the control group emphasized the necessity of evaluation of the difference between PTC and non-neoplastic tissue and 4.5 was the suggested cut-off for the difference between the scores of these 2 groups (24). The criteria for positivity were staining with any intensity in any percentage of cells. Huang et al used minimum 10% positive-cells criteria for inclusion in the positive CK19 record. They reported a significant difference between PTC and non-neoplastic pathologies in CK19 expression (26). Liu et al observed a significant difference between PTC and benign tissues in expressions of CK19, which was the most sensitive marker, used, but had the lowest specificity. In the present study, similar findings were observed and staining in at least 1% of the cells was found in all PTC and non-neoplastic tissues. To improve the specificity of CK19, a quantitative scoring system was used and PTC and non-neoplastic tissue scores were compared. Due to the diversity of the staining patterns of non-neoplastic tissues in other studies, in this study, both intensity and volume of positive cells were considered in the proposed scoring system. The suggested cut-off with 95% sensitivity and 72% specificity was 5.5, which is quite high. Despite the nearly ideal sensitivity and a marked increase in specificity, the specificity could be improved if the PTC score and the non-neoplastic score were compared. The proposed cut-off for the difference between these 2 types of tissue was 1.5 with 100% sensitivity and specificity. This is rather low and impractical if used as an individual marker (22). Galectin-3 expressions are also controversial and some studies have reported a significant absence of this marker in nodular goiter or Hashimoto thyroiditis (26). On the other hand, many reports contain varying degrees of positivity in non-PTC entities (14-16). These findings imply that for using this marker as a single tool for diagnosis, it should be used properly with scoring both the intensity and the volume of positivity. Results of the current work showed a 2.5 value for the cut-off score to diagnose the tissue as PTC, and both sensitivity and specificity were high, being 98% and 99%, respectively. The value of 3.5 was suggested for the cut-off difference between the scores of the 2 tissue types with 100% sensitivity and specificity in diagnosis of PTC. This revealed a rather great difference between the staining patterns of the 2 groups, and thus galectin-3 can be used as an individual marker distinguishing non-neoplastic tissue from PTC cells. HBME-1, a marker named after its expression by mesothelial cells, is also used in many studies. Most studies showed a high sensitivity and intermediate specificity for this marker when only its positivity mattered (23-25). Finally, the combination of IHC markers can improve both sensitivity and specificity. Using a combination of

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CK19 and HBME-1, compared with using each alone, improves sensitivity and specificity (22). On the other hand, in Cho et al study, using all 4 markers of HBME-1, CK19, galectin-3, and CD56 improved specificity but lowered sensitivity drastically (25). The results of Murtezaoglu et al showed that using HBME-1, CK19, TROP-2, and galectin-3 improved sensitivity but decreased specificity. These contradicting findings were the result of not using a uniform method for IHC interpretation in PTC. Here, the total score was calculated for combination of 2 markers as well as all the 3 markers used in this study. The most sensitive combination was CK19 and galectin-3, and the most specific combination was HBME-1 and galectin-3. The suggested total score for using all the 3 markers was 11.5 with 99% sensitivity and 100% specificity (24). This study only included the non-neoplastic tissue while excluding any pathologic findings, and the areas of Hashimoto thyroiditis or other sources of diagnostic difficulties were excluded as well. This was the main limitation of the current study and thus further studies are suggested to evaluate this scoring system in other entities like Hashimoto thyroiditis.

Conclusion

Due to some degree of CK19, HBME-1, and galectin-3 IHC staining observed in non-neoplastic tissue, diagnosis of PTC cannot be achieved by mere positive/negative interpretation. A proper scoring system can solve this problem. HBME-1 and galectin-3 can be used individually while their combination is very specific for PTC diagnosis. Higher-level studies with greater sample sizes are required to achieve more reliable results. Moreover, the staining score can be separately calculated for each non-neoplastic diagnosis, such as Hashimoto and goiter, or other diagnoses.

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Conflict of Interests

The authors declare that they have no competing interests.

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