Value of cluster of differentiation 56, Hector Battifora mesothelial-1, and cytokeratin 19 expression in predicting the risk of papillary thyroid carcinoma occurrence in Hashimoto's thyroiditis patients, which will advise early total thyroidectomy in those patients

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Background
There were no previous studies that have tried to find the prediction of papillary thyroid carcinoma (PTC) occurrence in Hashimoto's thyroiditis (HT) that will advise early thyroidectomy in HT cases with high risk of progression to PTC.
We aimed to use a panel of cluster of differentiation 56 (CD56), Hector Battifora mesothelial-1 (HBME-1), and cytokeratin 19 (CK19) to detect their predictive ability for HT progression into PTC.
Patients and methods
We included five groups of paraffin blocks that were retrieved from 70 patients. The first group included 20 cases of PTC, the second group included 20 samples from the same cases previously diagnosed as HT, the third group included 30 cases of HT, the fourth group included 30 samples from the same cases previously diagnosed as HT, and the fifth group had 20 cases of PTC without a history of HT. The sections were stained by CD56, HBME-1, and CK19 using immunohistochemistry.
Results
There is a significant difference between the second (HT that will be transformed to PTC) and the fourth (HT that will not be transformed to PTC) groups as regards CD56, HBME-1, and CK19 expression (P=0.012).
For the differentiation between HT that will be transformed to PTC from HT that will not be transformed to PTC, negative CD56 expression was of highest sensitivity (90%) and diffuse positive HBME-1 expression was of highest specificity (95.7%).
Conclusion
A combination of negative CD56 expression and diffuse positive HBME-1 could be used with high sensitivity and specificity in predicting PTC occurrence in certain cases of HT and these patients will be advised to early total thyroidectomy to avoid PTC occurrence in the future.

Keywords:
cluster of differentiation 56, cytokeratin 19, Hashimoto's, thyroiditis, Hector Battifora mesothelial-1, immunohistochemistry, papillary thyroid carcinoma, total thyroidectomy

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Introduction

The most common thyroid malignancy is the papillary thyroid carcinoma (PTC) that forms about 80% of thyroid malignancies [1]. The most common autoimmune disease of the thyroid gland is Hashimoto's thyroiditis (HT) which the commonest cause of hypothyroidism [2]. The association and pathogenic relations between HT and PTC remains controversial and still need further qualifications [3]. Dailey *et al.* [4] first described that there is a relationship between both thyroid lesions in 1955 and since then, there are many researchers who have tried to cover such scientific point but they provided conflicting results. It is essential also to mention that molecular analyses have indicated that PTC had a high amount of lymphocytic infiltration that suggested the role of immunological factors in malignant progression [5,6]. The management of HT patients is mostly conservative with L-thyroxine [7]. Moreover performing total thyroidectomy is not preferred for such patients due to

the presence of inflammatory response that surrounds the thyroid gland and leads to more difficult surgical resection [8]. There are few indications for early surgical intervention in HT patients, for example worsening of clinical symptoms that are related to the disease, malignancy suspicion, or a goiter with marked increase in size [8–10]. Although a plethora of studies have identified the indications of surgery in patients with HT [11], performing early thyroidectomy is still a controversial method for their management. So it would be beneficial to use the available biomarkers to predict the cases of HT that will have a high liability of progression to PTC, which will be helpful in performing early thyroidectomy for those patients even if there are no marker disfigurement or marked pressure symptoms.

An increasing number of promising biomarkers have emerged for differentiation between benign and malignant thyroid lesions, for example cluster of differentiation 56 (CD56), Hector Battifora mesothelial (HBME-1), and cytokeratin 19 (CK19). CD56 is an adhesion molecule that is present in the neural cells [12]. It is expressed in normal non-neoplastic thyroid follicular cells with decreased expression in thyroid malignancies mainly PTC [13]. HBME-1 exists in the microvilli of tracheal epithelium and mesothelial cells [14]. There are many previous studies that have clarified the role of HBME-1 expression in thyroid malignancies like PTC [15]. CK19 is an intermediate filament protein of type I that is widely expressed in normal epithelial cells [12]. A lot of researchers have clarified its strong and diffuse positivity in PTC [16]. Many of all those studies have assessed only the expression of a single marker of them in benign and malignant thyroid lesions, but only few of them have explored the value of their combined expression [17,18]. Moreover, most studies have assessed the roles of such markers in PTC diagnosis or to distinguish HT from PTC, but we noticed that there are no previous study which tried to assess the value of using such markers in the prediction of PTC occurrence in HT patients that will advise performing early thyroidectomy in HT cases with high risk of progression to PTC before its occurrence which will subsequently decrease the malignancy risk in such patients.

Therefore, we aimed in the present study to evaluate the usefulness of using a panel of the most sensitive and specific markers for PTC, as previously mentioned, for example CD56, HBME-1, and CK19 individually and in combination, to detect their ability to differentiate HT from PTC and detect their value in the prediction of malignant progression of certain cases of HT to PTC.

Patients and methods

In the period between January 2012 and December 2016, 200 consecutive patients with either solitary thyroid nodule or multinodular goiter underwent total thyroidectomy in the General Surgery Hospital, Oncology Unit, Faculty of Medicine, Zagazig University and El Mansura University. We include in our study 70 patients that had a previous history of subtotal thyroidectomy. Our cases were divided into 20 cases of PTC with a previous history of HT, 20 cases of PTC without a previous history of HT, and 30 cases that was diagnosed as HT in the Pathology Department, Faculty of Medicine, Zagazig University. Data from all patients were retrospectively obtained from the files of the shared departments. All the 70 thyroidectomy samples are processed and subjected to routine hematoxylin and eosin stain. We ordered all cases to bring their paraffin blocks that was acquired by subtotal thyroidectomy to do our research on them and on blocks retrieved from the total thyroidectomy samples. In such a method the results classification comprises five groups. First group – 20 paraffin blocks of total thyroidectomy specimen that were recently diagnosed as PTC. Second group – 20 paraffin blocks of the same cases historically diagnosed as HT by subtotal thyroidectomy since variable periods. Third group – 30 paraffin blocks of total thyroidectomy specimen that were recently diagnosed as HT. Fourth group – 30 paraffin blocks of the same cases historically diagnosed as HT by subtotal thyroidectomy since variable periods. Fifth group – 20 paraffin blocks of that were diagnosed as PTC without a previous history of HT.

The collection and subsequent analysis of patients' data was duly approved by the IRB Committee in Faculty of Medicine, Zagazig University. The gross and histopathological features of each case were evaluated independently by two pathologists before arriving at the final diagnosis. We used the tumor, node, and metastasis staging system modified by the American Joint Committee on Cancer – Cancer Staging, seventh edition for surgical staging of PTC [19].

Immunohistochemical staining

Sections from paraffin blocks that were retrieved from the five groups were deparaffinized in xylene and rehydrated in absolute alcohol. Antigen retrieval in citrate buffer was used after the sections were treated in a microwave at 8 W for 6 min, and the sections were then left to cool for 20 min.

Peroxidase blocks were done. After that we incubated the slides with the primary anti-CD56 (clone 123C3, 1 : 100; DakoCytomation, Glostrup, Denmark); anti-HBME-1 (clone HBME-1, 1 : 50; DakoCytomation), and anti-CK19 (1 : 100; DakoCytomation) antibodies at room temperature, and then washed them lightly in PBS, at a pH of 7.6. Subsequent to this incubation with the secondary biotin-conjugated antibody for 1 h was done and then with peroxidase-conjugated streptavidin. Diaminobenzidine tetrachloride was added for 25 min, and finally the slides are counterstained in hematoxylin, then we dehydrated the slides, cleaned, and mounted them [20]. Positive and negative control slides were included. Positive controls were sections from neuroblastoma, mesothelioma, and skin for CD56, HBME-1, and CK19, respectively. Negative controls were done by the removal of primary antibodies and their replacement with PBS [21].

Interpretation of immunohistochemical (IHC) staining of the studied markers:

(1) Membranous expression with or without cytoplasmic staining of the cells qualified the case as positive for CD56 and CK19 [16,21].
(2) Cytoplasmic expression with or without membranous staining of the cells qualified the case as positive for HBME-1 [22].

Scoring for the immunomarkers by semiquantitative assessment of marker expression:

(1) For all antibodies, immunoreactivity was considered positive if more than 10% of follicular epithelial cells were stained [32].
(2) The immunoreactivity was scored as negative, focally positive (+: <25%), positive (++: 25–50%), or diffusely positive (+++: >50%), based on the extent of the reaction [21–23].

Statistical analysis

Quantitative data were expressed as the mean±SD and median (range), and qualitative data were expressed as absolute frequencies (number) and relative frequencies (percentage). Categorical data were compared using χ^2-test or Fisher's exact test when appropriate. Paired categorical variables were compared using McNemar's test. All tests were two sided. A P value of less than 0.05 was considered statistically significant. Validity IHC was calculated using diagnostic performance depending on sample 2×2 contingency tables generation using the histological examination as the reference (gold) standard. The sensitivities, specificities, positive predictive values, negative predictive values, and accuracies, with their respective 95% confidence intervals were calculated. All data were collected, tabulated, and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, Illinois, USA) and MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium).

Results

Patients criteria

The clinical data of the patients are summarized in Table 1

Our study included 10 (14%) men and 60 (86%) women with age ranged from 29 to 51 years for patients with PTC with a history of HT, 29–52 years for patients with PTC without a history of HT, and 22–40 years for patients with HT.

(1) First group: 20 paraffin blocks of total thyroidectomy specimen that were recently diagnosed as PTC, included two (10%) men and 18 (90%) women.
(2) Second group: 20 paraffin blocks of the same cases historically diagnosed as HT by subtotal thyroidectomy for variable periods.
(3) Third group: 30 paraffin blocks of total thyroidectomy specimen that were recently diagnosed as HT included three (10%) men and 27 (90%) women.
(4) Fourth group: 30 paraffin blocks of the same cases historically diagnosed as HT by subtotal thyroidectomy for variable periods.
(5) Fifth group: 20 paraffin blocks that were diagnosed as PTC without a previous history of HT included five (25%) men and 15 (75%) women.

Immunohistochemical expression in the studied thyroid

CD56 expression in the studies lesions

Among the first group, negative CD56 expression was detected in 16 (80%), focal positive CD56 expression was observed in two (10%), and diffuse positive CD56 expression was found in two (10%) of the cases of PTC that was on top of HT (Tables 1 and 2, Figs. 1e, f, 2e and 3c.

Among the second group, negative CD56 expression was detected in 10 (50%), focal positive CD56 expression was observed in eight (40%), and diffuse positive CD56 expression was found in two (10%) of the cases of HT that were found to be transformed into PTC later on.

Among the third group, negative CD56 expression was detected in five (16.7%), focal positive CD56

Table 1 Comparison between studied groups as regards demographic and immunohistochemical staining

				P value		
	PTC with HT (*N*=20)	PTC without HT (*N*=20)	HT (*N*=30)	P_1	P_2	P_3
Sex						
Male	2 (10)	5 (25)	3 (10)			
Female	18 (90)	15 (75)	27 (90)			
Age (years)						
Mean±SD	40.75±7.12	41.20±8.04	31.06±4.89			
Median (range)	38.50 (29–51)	38.50 (29–52)	30 (22–40)			
HBME-1 (historical HT)						
Negative	4 (20)		22 (73.3)	<0.001[a]		
Focal positive	8 (40)		7 (23.3)			
Diffuse positive	8 (40)		1 (3.3)			
HBME-1 (recent)						
Negative	2 (10)	3 (15)	22 (73.3)	<0.001[a]	0.619[a]	<0.001[a]
Focal positive	4 (20)	6 (30)	7 (23.3)			
Diffuse positive	14 (70)	11 (55)	1 (3.3)			
P value	0.031[b]		1.000[b]			
CK19 (historical HT)						
Negative	4 (20)		21 (70)	<0.001[a]		
Focal positive	5 (25)		7 (23.3)			
Diffuse positive	11 (55)		2 (6.7)			
CK19 (recent)						
Negative	3 (15)	5 (25)	21 (70)	<0.001[a]	0.717[a]	<0.001[a]
Focal positive	4 (20)	4 (20)	7 (23.3)			
Diffuse positive	13 (65)	11 (55)	2 (6.7)			
P value	0.375[b]		1.000[b]			
CD56 (historical HT)						
Negative	10 (50)		5 (16.7)	0.012[a]		
Focal positive	8 (40)		12 (40)			
Diffuse positive	2 (10)		13 (43.3)			
CD56 (recent)						
Negative	16 (80)	17 (85)	5 (16.7)	<0.001[a]	0.834[a]	<0.001[a]
Focal positive	2 (10)	2 (10)	12 (40)			
Diffuse positive	2 (10)	1 (5)	13 (43.3)			
P value	0.070[b]		1.000[b]			

Categorical variables were expressed as number (percentage); continuous variables were expressed as mean±SD and median (range); CD56, cluster of differentiation 56; CK19, cytokeratin 19; HBME-1, Hector Battifora mesothelial-1; HT, Hashimoto's thyroiditis; PTC, papillary thyroid carcinoma; P_1, papillary thyroid carcinoma with Hashimoto's thyroiditis versus Hashimoto's thyroiditis; P_2, papillary thyroid carcinoma with Hashimoto's thyroiditis versus papillary thyroid carcinoma without Hashimoto's thyroiditis; P_3, papillary thyroid carcinoma without Hashimoto's thyroiditis versus Hashimoto's thyroiditis; [a]χ^2-test; [b]McNemar's test; $P<0.05$, significant.

expression was observed in 12 (40%), and diffuse positive CD56 expression was found in 13 (43.3%) of cases of HT that was not transformed into PTC later on.

Among the fourth group, negative CD56 expression was detected in five (16.7%), focal positive CD56 expression was observed in 12 (40%), and diffuse positive CD56 expression was found in 13 (43.3%) of cases of HT that was not transformed into PTC later on.

Among the fifth group, negative CD56 expression was detected in 17 (85%), focal positive CD56 expression was observed in two (10%), and diffuse positive CD56 expression was found in one (5%) of the cases of PTC with no history of HT.

HBME-1 expression in the studied lesions HBME-1 signal was detected predominantly in the cytoplasm

Among the first group, negative HBME-1 expression was detected in two (10%), focal positive HBME-1 expression was observed in four (20%), and diffuse positive HBME-1 expression was found in 14 (70%) of cases of PTC that was on top of HT (Tables 1 and 3, Figs. 1a, b, 2a, b and 3a).

Among the second group, negative HBME-1 expression was detected in four (20%), focal positive HBME-1 expression was observed in eight (40%), and diffuse positive HBME-1 expression was found in eight (40%) of cases of HT that were found to be transformed into PTC later on.

Among the third group, negative HBME-1 expression was detected in 22 (73.3%), focal positive HBME-1

Table 2 Change in cluster of differentiation 56 immunohistochemistry between historical specimen and recent specimen

CD56 IHC in historical specimen	CD56 IHC in recent specimen			Total
	Negative	Focal positive	Diffuse positive	
PTC with HT				
Negative	10 (50)	0 (0)	0 (0)	10 (50)
Focal positive	6 (30)	1 (5)	1 (5)	8 (40)
Diffuse positive	0 (0)	1 (5)	1 (5)	2 (10)
Total	16 (80)	2 (10)	2 (10)	20 (100)
HT				
Negative	5 (16.7)	0 (0)	0 (0)	5 (16.7)
Focal positive	0 (0)	12 (40)	0 (0)	12 (40)
Diffuse positive	0 (0)	0 (0)	13 (43.3)	13 (43.3)
Total	5 (16.7)	12 (40)	13 (43.3)	30 (100)

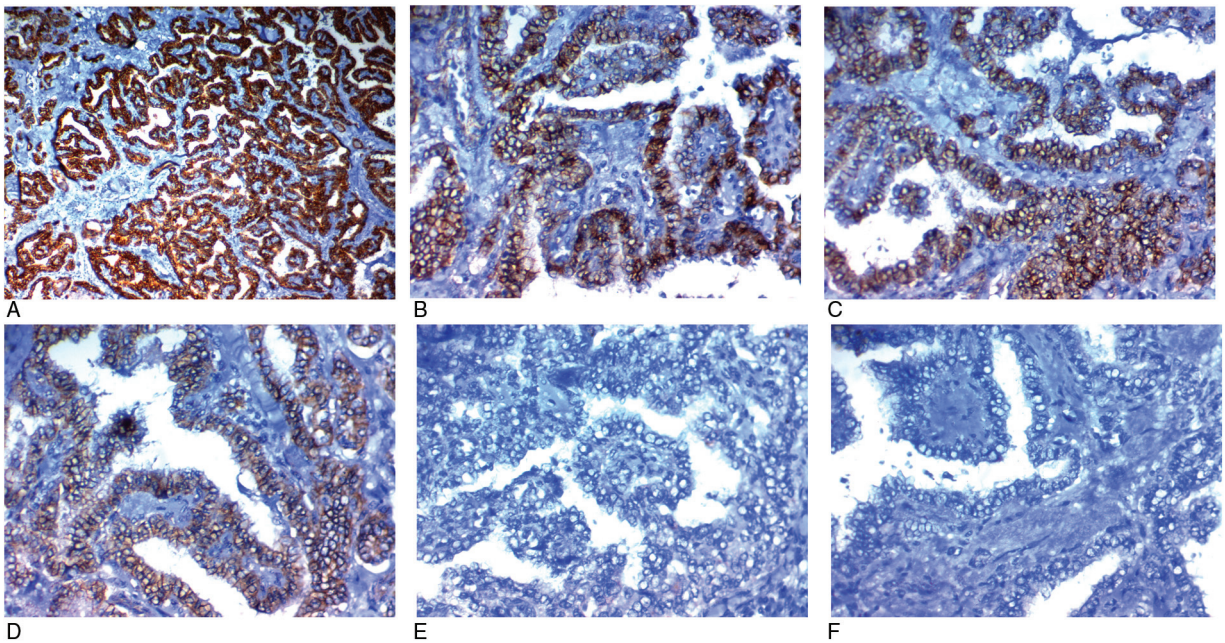
Categorical variables were expressed as number (percentage); CD56, cluster of differentiation 56; HT, Hashimoto's thyroiditis; IHC, immunohistochemistry; PTC, papillary thyroid carcinoma.

Table 3 Change in Hector Battifora mesothelial-1 immunohistochemistry between historical specimen and recent specimen

HBME-1 IHC in historical specimen	HBME-1 IHC in recent specimen			Total
	Negative	Focal positive	Diffuse positive	
PTC with HT				
Negative	2 (10)	0 (0)	2 (10)	4 (20)
Focal positive	0 (0)	4 (20)	4 (20)	8 (40)
Diffuse positive	0 (0)	0 (0)	8 (40)	8 (40)
Total	2 (10)	4 (20)	14 (70)	20 (100)
HT				
Negative	22 (73.3)	0 (0)	0 (0)	22 (73.3)
Focal positive	0 (0)	7 (23.3)	0 (0)	7 (23.3)
Diffuse positive	0 (0)	0 (0)	1 (3.3)	1 (3.3)
Total	22 (73.3)	7 (23.3)	1 (3.3)	30 (100)

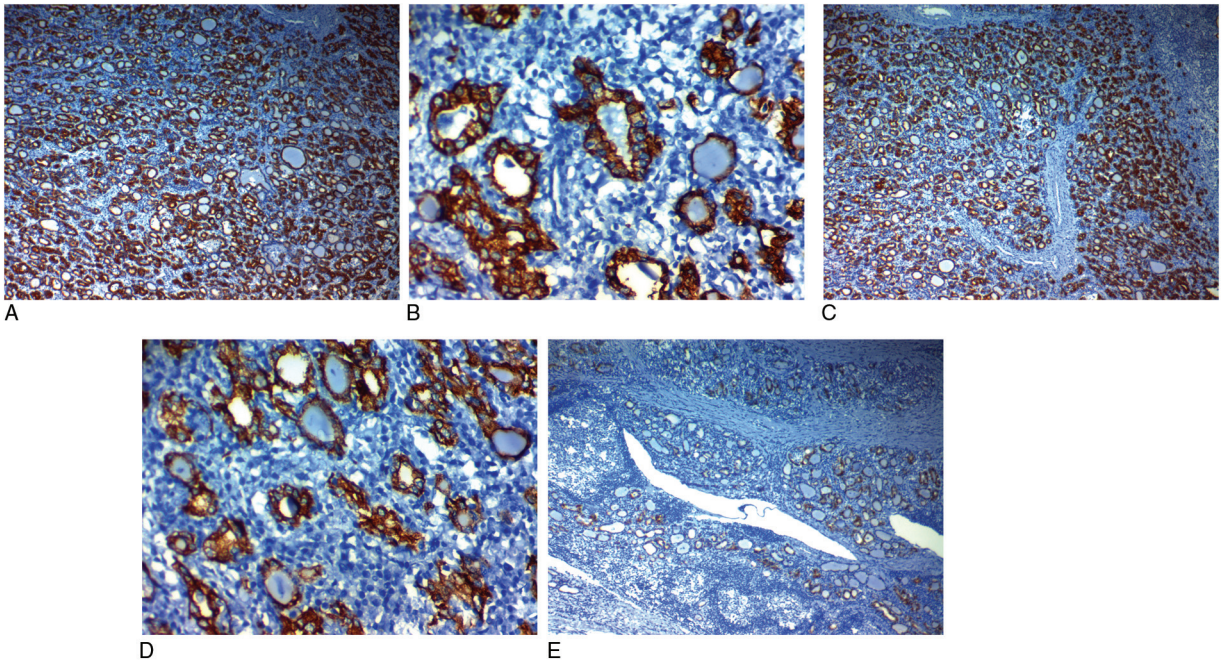
Categorical variables were expressed as number (percentage); HBME-1, Hector Battifora mesothelial-1; HT, Hashimoto's thyroiditis; IHC, immunohistochemistry; PTC, papillary thyroid carcinoma.

Figure 1



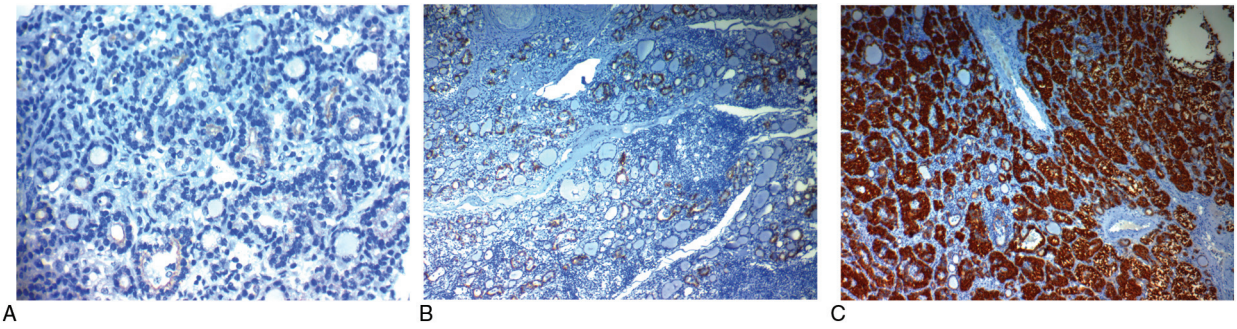
Papillary thyroid carcinoma immunohistochemistry. (a) PTC on top of HT showed diffuse positive HBME-1 expression (×100). (b) PTC without previous history of HT showed diffuse positive HBME-1 expression (×400). (c) PTC on top of HT showed diffuse positive CK19 expression (×400). (d) PTC without previous history of HT showed diffuse positive CK19 expression (×400). (e) PTC on top of HT showed negative CD56 expression (×400). (f) PTC without previous history of HT showed negative CD56 expression (×400). CD56, cluster of differentiation 56; CK19, cytokeratin 19; HBME-1, Hector Battifora mesothelial-1; HT, Hashimoto's thyroiditis; PTC, papillary thyroid carcinoma.

Figure 2



Hashimoto's thyroiditis that was transformed to papillary thyroid carcinoma immunohistochemical expression. (a) Diffuse positive Hector Battifora mesothelial-1 expression (×100). (b) High power of the previous image showed diffuse positive Hector Battifora mesothelial-1 expression (×400). (c) Diffuse positive cytokeratin 19 expression (×100). (d) High power of the previous image showed diffuse positive cytokeratin 19 expression (×400). (e) Negative cluster of differentiation 56 expression (×400).

Figure 3



Hashimoto's thyroiditis that was transformed to papillary thyroid carcinoma immunohistochemical expression. (a) Negative Hector Battifora mesothelial-1 expression (×400). (b) Negative cytokeratin 19 expression (×100). (c) Diffuse positive cluster of differentiation 56 expression (×100).

expression was observed in seven (23.3%), and diffuse positive HBME-1 expression was found in one (3.3%) of cases of HT that was not transformed into PTC later on.

Among the fourth group, negative HBME-1 expression was detected in 22 (73.3%), focal positive HBME-1 expression was observed in seven (23.3%), and diffuse positive HBME-1 expression was found in one (3.3%) of cases of HT that was not transformed into PTC later on.

Among the fifth group, negative HBME-1 expression was detected in three (15%), focal positive HBME-1 expression was observed in six (30%), and diffuse positive HBME-1 expression was found in 11 (55%) of cases of PTC with no history of HT.

CK19 expression in the studied lesions CK19 expression was detected in the cell membrane with or without the cytoplasm

Among the first group, negative CK19 expression was detected in three (15%), focal positive CK19 expression was observed in four (20%), and diffuse positive CK19 expression was found in 13 (65%) of cases of PTC that was on top of HT (Tables 1 and 4, Figs. 1c, d, 2c, d and 3b).

Among the second group, negative CK19 expression was detected in four (20%), focal positive CK19

Table 4 Change in cytokeratin 19 immunohistochemistry between historical specimen and recent specimen

CK19 IHC in historical specimen	CK19 IHC in recent specimen			Total
	Negative	Focal positive	Diffuse positive	
PTC with HT				
Negative	3 (15)	1 (5)	0 (0)	4 (20)
Focal positive	0 (0)	2 (10)	3 (15)	5 (25)
Diffuse positive	0 (0)	1 (5)	10 (50)	11 (55)
Total	3 (15)	4 (20)	13 (65)	1 (100)
HT				
Negative	21 (70)	0 (0)	0 (0)	21 (70)
Focal positive	0 (0)	7 (23.3)	0 (0)	7 (23.3)
Diffuse positive	0 (0)	0 (0)	2 (6.7)	2 (6.7)
Total	21 (70)	7 (23.3)	2 (6.7)	30 (100)

Categorical variables were expressed as number (percentage); CK19, cytokeratin 19; HT, Hashimoto's thyroiditis; IHC, immunohistochemistry.

expression was observed in five (25%), and diffuse positive CK19 expression was found in 11 (55%) of cases of HT that were found to be transformed into PTC later on (Fig. 1a).

Among the third group, negative CK19 expression was detected in 21 (70%), focal positive CK19 expression was observed in seven (23.3%), and diffuse positive CK19 expression was found in two (6.7%) of cases of HT that was not transformed into PTC later on.

Negative CK19 expression was detected in 21 (70%), focal positive CK19 expression was observed in seven (23.3%), and diffuse positive CK19 expression was found in two (6.7%) of cases of HT that was not transformed into PTC later on. Among the fifth group, negative CK19 expression was detected in five (25%), focal positive CK19 expression was observed in four (20%), and diffuse positive CK19 expression was found in 11 (55%) of cases of PTC with no history of HT.

No statistically significant difference was found between the first and fifth groups, second and fifth groups, third and fourth groups as regards all marker expressions and (first and second groups as regards CD56 and CK19 expression.

There is a highly significant statistical difference found between the second and fourth groups as regards CD56, HBME-1, and CK19 expression (P=0.012, 0.000, respectively).

There is a significant statistical difference found between the first and the second group as regards HBME-1 expression (P=0.031).

There is a highly significant statistical difference that was found between the first and fourth groups, first and third groups, fifth and third groups, and fifth and fourth group as regards all marker expressions (P=0.000).

Specificity and sensitivity of each marker

Diagnostic validity of CD56 was of highest sensitivity (90%) in differentiating HT that will be transformed to PTC from HT that will not be transformed to PTC (Table 5).

Diagnostic validity of HBME-1 was of highest specificity in differentiating HT that will be transformed to PTC from HT that will not be transformed to PTC.

Discussion

A plethora of researchers have assessed the association between HT and PTC, some of them found a significant positive correlation [2,3], but others have not found any correlation between both conditions [24]. The importance of finding such association is that the presence of goiter of any size in a patient with HT should raise the possibility of developing PTC later on, and also indicated the need for deeper investigations to exclude the coexistence of malignancy in such cases.

The most recent hypothesis that we tried to prove here in our study is if we can predict the liability for malignant progression of certain cases of HT to PTC that will be of great help to the patient as we will advise the surgeon to do early total thyroidectomy for those HT cases with more liability for malignant progression.

Akhtar and Scognamiglio [25] found that HT and PTC have the same pluripotent stem cell origins, which proved that the association between conditions is antibody specific and may have an oncogenic role [26]. It was found that the elevated

Table 5 Validity of Hector Battifora mesothelial-1, cytokeratin 19, and cluster of differentiation 56 immunohistochemistry

Groups	IHC	TP	FP	TN	FN	SN	SP	Accuracy	PPV	NPV
HT: (1) transformed to PTC vs. HT (2) that is not transformed to PTC	HBME-1 (diffuse=HT (1))	14	1	29	6	65 (49.9–90.1)	95.7 (90.2–100)	80 (76.4–95.6)	93.3 (80.7–100)	82.9 (70.4–95.3)
	CK19 (diffuse=HT (1))	13	2	28	7	60 (44.1–85.9)	83.3 (84.4–90)	82 (71.4–90.6)	86.7 (69.5–100)	80 (66.7–93.3)
	CD56 (negative/focal=HT (1))	18	17	13	2	90 (76.9–100)	40 (25.6–55)	50 (48.5–70.5)	51.4 (34.9–68)	86.7 (38.9–74.4)
PTC vs. HT (1) same patients	HBME-1 (diffuse=PTC)	14	9	11	6	70 (49.9–90.1)	55 (33.2–76.8)	62.5 (47.5–77.5)	60.9 (40.9–80.8)	64.7 (42–87.4)
	CK19 (diffuse=PTC)	13	11	9	7	65 (44.1–85.9)	45 (23.2–66.8)	55 (39.6–70.4)	54.2 (34.2–74.1)	56.3 (31.9–80.6)
	CD56 (negative/focal=PTC)	18	18	2	2	90 (76.9–100)	10 (0–23.1)	50 (34.5–65.5)	50 (33.7–66.3)	50 (1–99)
PTC vs. HT (2)	HBME-1 (diffuse=PTC)	14	1	29	6	70 (49.9–90.1)	96.7 (90.2–100)	86 (76.4–95.6)	93.3 (80.7–100)	82.9 (70.4–95.3)
	CK19 (diffuse=PTC)	13	2	28	7	65 (44.1–85.9)	93.3 (84.4–100)	82 (71.4–92.6)	86.7 (69.5–100)	80 (66.7–93.3)
	CD56 (negative/focal=PTC)	18	17	13	2	90 (76.9–100)	43.3 (25.6–61.1)	52 (48.5–75.5)	51.4 (34.9–68)	86.7 (38.9–74.4)

CD56, cluster of differentiation 56; CK19, cytokeratin 19; FN, false negative; FP, false positive; HBME-1, Hector Battifora mesothelial-1; HT, Hashimoto's thyroiditis; NPV, negative predictive value HT that is transformed into PTC=(1); HT that is not transformed into PTC=(2); PPV, positive predictive value; PTC, papillary thyroid carcinoma; SN, sensitivity; SP, specificity; TN, true negative; TP, true positive.

levels of TSH in HT patients could be also be risk factors for cancer [27].

So many previous researchers have tried to differentiate PTC from HT by IHC and have succeeded in such issue using certain available sensitive and specific markers, but there are no previous study that tried to use such IHC markers to predict the progression of HT to PTC, which could identify a group of patients that will be in a certain need to do early total thyroidectomy for the management of HT to avoid malignant transformation into PTC later on.

CD56 has been found to be related to follicular epithelium differentiation, and many previous authors reported high CD56 expression in normal non-neoplastic thyroid tissue and some benign thyroid lesions [13,28]. In accordance with those studies, we currently report a high positive CD56 expression in 83.3% of HT cases that found to not transform to PTC. On the other hand, negative CD56 expression was observed in 80–85% of PTC that has occurred on top of HT and that has occurred *de novo*, respectively, and we have detected highly significant difference between PTC and HT cases that was not transformed to PTC ($P<0.001$). Similarly, previous studies reported negative CD56 expression in most of their studied PTC cases [28,29].

We found that negative CD56 expression was detected in 10 (50%); focal positive CD56 expression was observed in eight (40%), and diffuse positive CD56 expression was found in two (10%) of cases of HT that found to be transformed into PTC later on. There was no statistically significant difference between CD56 expressions in PTC cases and HT cases that was transformed into PT later on, but CD56 distinguished the HT group that did not transform into PTC later from the HT group that was transformed into PTC later, so it can be used to categorize HT into cases with high incidence of malignant transformation into PTC and cases with low in incidence of such transformation. The sensitivity and the specificity for CD56 in distinguishing the HT group that did not transform into PTC later from the HT group that was transformed into PTC later were 90 and 40%, respectively, so CD56 was of highest sensitivity as a negative marker in differentiating HT that will be transformed to PTC from HT that will not be transformed to PTC which will be very helpful to us in the prediction of cases with high incidence of malignant transformation into PTC and cases with low incidence of such transformation.

HBME-1 is a component of the microvilli that is located on the surface of mesothelial cells [15]. Previous studies demonstrated that HBME-1 overexpression as detected by IHC, was observed in thyroid cancers and it was a sensitive marker for PTCs [30,31].

That was similar to us as we proved that HBME-1 was positive in 85–90% of cases of PTC that has occurred *de novo* and that has occurred on top of HT, respectively, whereas it was negative in most cases of HT (73.3%) that was not transformed to PTC later on and we have detected a highly significant difference between PTC and HT cases that was not transformed to PTC ($P<0.001$). Our results proved that HBME-1 has been reported to be one of the most promising markers [17,32,33] of HBME-1 positivity in 70% classic PTC, and Prasad *et al.* [17] demonstrated HBME-1 expression in 85% PTC. This was slightly different from Arturs *et al.* [29] who detected negative HBME-1 expression in all benign lesions, whereas they observed its positive expression in all cases of PTC.

In our study when we used HBME-1 in the prediction of HT to PTC, we found that the sensitivity and the specificity for HBME-1 in distinguishing the HT group that did not transform into PTC later from the HT group that was transformed into PTC later were 65 and 95.7%, respectively, so HBME-1 was of highest specificity in differentiating HT that will be transformed to PTC from HT that will not be transformed to PTC that will be very helpful to us in the prediction of cases with high incidence of malignant transformation into PTC and cases with low incidence of such transformation, and this was in agreement with Husain *et al.* [16], who showed that HBME-1 was a sensitive and specific marker to differentiate benign from malignant lesions which was higher than any markers.

We found that negative HBME-1 expression was detected in four (20%); focal positive HBME-1 expression was observed in eight (40%), and diffuse positive HBME-1 expression was found in eight (40%) of the cases of HT that were found to be transformed into PTC later on, and there was no statistically significant difference between HBME-1 expression in PTC cases and HT cases that was transformed into PT later on, but HBME-1 expression distinguished the HT group that did not transform into PTC later from the HT group that was transformed into PTC later, so it can be used to categorize HT into cases with high incidence of malignant transformation into PTC and cases with low incidence of such transformation.

CK19 (keratin 19) is a keratin family member that plays an essential role in the structure and integrity of most epithelial cells, but its role in the diagnosis of PTC is still a point of research [24–36].

Some studies have found that negative CK19 expression was found in all benign thyroid lesions [18], whereas Cheung *et al.* [33] demonstrated that 20% of benign thyroid lesions were focally CK19 positive. The study by Nasr *et al.* [32] also noted a 68% CK19 positivity in benign lesions, but staining intensity was weak. In all these cases, CKI9 staining was patchy and moderate. Zhu *et al.* [37] suggested that CK19 was not a specific marker of PTC.Sahoo *et al.* [38] and Guyetant *et al.* [39] have demonstrated that all cases of PTC showed strong CK19 positivity.

Prasad *et al.* [17] showed a high sensitivity and specificity of CK19 in PTC, so the chief benefit of CK19 lies in its diagnostic ability of PTC and its negative staining is a sign against PTC. Negative staining for CK19, therefore, is strong evidence against PTC. We have proved results similar to most of these previous studies as we found that CKI9 was positive in 75–85% of cases of PTC that has occurred *de novo* and that has occurred on top of HT, respectively, whereas it was negative in most cases of HT (70%) that was not transformed to PTC later on and we have detected a highly significant difference between PTC and HT cases that was not transformed to PTC ($P<0.001$).

The sensitivity and the specificity for CK19 in distinguishing the HT group that did not transform into PTC later from the HT group that was transformed into PTC later were 60 and 83.3%, respectively.

CH-19 was of moderate sensitivity and specificity in differentiating HT that will be transformed to PTC from HT that will not be transformed to PTC that will be also helpful in addition to HBME-1 and CD56 to us in the prediction of cases with high incidence of malignant transformation into PTC.

In contrast to our results that HT increased the risk of PTC occurrence, Segal *et al.* [40] suggest that HT does not increase but rather delays PTC occurrence due to the presence of circulating antibodies which may be a significant factor which could prevent cancer development and also hinder nodal metastases in transformed cases. These results are in contrast to the investigations by Di Pasquale *et al.* [41], who proved a strong autoimmune background in all cases of PTC coexisting with HT. So further studies are needed to prove and clarify our results.

In summary

(1) The most common thyroid malignancy is PTC and the most common autoimmune disease of the thyroid gland is HT.
(2) The association and pathogenic relations between both HT and PTC remains controversial.
(3) ?Dailey and colleagues first described that there is a relationship between both the thyroid lesions and since then, there are many conflicting results regarding such issue.
(4) Management of HT patients is mostly conservative and performing total thyroidectomy is not preferred due to the presence of inflammatory response that surrounds the thyroid gland which can lead to more difficult surgical resection.
(5) Although a plethora of studies have identified the indications of surgery in patients with HT, performing early thyroidectomy is still a controversial method for their management.
(6) It would be beneficial to use the available biomarkers to predict the cases of that HT will have a high liability of progression to PTC which will be helpful in performing early thyroidectomy for those patients even if there is no marker disfigurement or marked pressure symptoms.
(7) We used the biomarkers that have emerged for differentiation between benign and malignant thyroid lesions, e.g. CD56, HBME-1, and CK19.
(8) Most studies have assessed the roles of such markers in PTC diagnosis or to distinguish HT from PTC, but we noticed that there are no previous studies which tried to assess the value of using such markers in the prediction of PTC occurrence in HT patients that will advise performing early thyroidectomy in HT cases with high risk of progression to PTC before its occurrence which subsequently will decrease the malignancy risk in such patients.

Conclusion

We detected that a panel of CD56 negative expression and HBME-1 diffuse expression is considered the most sensitive and specific for the prediction of PTC occurrence in certain HT cases.

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Conflicts of interest
There are no conflicts of interest.

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