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Application of Galectin-3, HBME-1, Cytokeratin-19, and CD56 in the Diagnosis of Thyroid Neoplasm

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Objective: The purpose of this study was to investigate the effectiveness of immunohistochemical thyroid malignancy markers Galectin-3, HBME-1, Cytokeratin-19, and CD56 in the diagnosis of thyroid neoplasm. **Methods:** The study included a total of 53 cases of thyroid neoplasm on which immunohistochemical IHC staining was performed using the manual staining method. **Results:** The histopathological diagnosis of thyroid nodules was papillary thyroid carcinoma in 40 (75.5%), follicular thyroid carcinoma in 3 (5.7%), follicular adenoma in 8 (15.1%), medullary thyroid carcinoma in 1 (1.9%), and Hurthle cell carcinoma in 1 (1.9%). Out of 40 cases of papillary thyroid carcinoma, Galectin-3 and CK19 were positive in 36 (90%) cases while expression of HBME1 was seen in 32 (80%) papillary thyroid carcinoma cases, and 16 (40%) stained positive for CD56. For the three follicular thyroid carcinoma cases, CD56 was positive in one case, but the marker was positive in 7 out of 8 cases (87.5%) of follicular adenoma. **Conclusion:** Our results suggest Galectin-3 and HBME-1 are the most sensitive diagnostic markers for differentiating papillary carcinoma from follicular neoplasm.

Keywords: Papillary thyroid carcinoma, Cytokeratin 19, Galectin-3, HBME-1, CD56.

Introduction

Thyroid nodules are common, and the purpose of evaluating them is to detect malignancy, which occurs in approximately 8%-15% of all nodules[1]. However, an increase in thyroid cancer incidence has been noted in reports in the last 30 years. Also, thyroid tumor cases have increased significantly in Mongolia over the past few years.

There are four key components to thyroid nodule assessment: clinical history and examination, serum thyroid stimulating hormone measurement, ultrasound and if indicated, fine-needle aspiration[2]. High-resolution ultrasound is recommended as the first line modality in the evaluation of thyroid nodules[3]. Fine-needle aspiration may be performed for nodules ≥ 1.0 cm depending on clinical and sonographic risk factors for thyroid cancer. Also, fine-needle aspiration specimens should be read by an experienced

cytopathologist and be reported according to the Bethesda Classification System. Surgery is indicated for FNA findings of malignancy or indeterminate cytology when there is a high-risk clinical context[2].

In the vast majority of cases, the pathological diagnosis of surgically removed thyroid nodules is possible by morphological examination in routine Hematoxylin-Eosin (H&E) stained sections. However, there are cases in which pathological criteria do not allow the differentiation between benign and malignant follicular-patterned thyroid tumors/lesions, making the distinction between these two groups quite subtle and challenging[4,5]. Ancillary tests, such as immunohistochemistry (IHC), can help distinguish thyroid lesions[6]. Several studies have shown that immunohistochemistry (IHC) may provide additional support in the evaluation and diagnosis of thyroid tumors/lesions[7-10].

For instance, Hector Battifora Mesothelial Cell-1 (HBME-1), Galectin-3 and Cytokeratin-19 (CK19) have been the most frequently used antibodies in thyroid pathology, although a wide range of sensibility and specificity values of these markers has been reported[3,6,11-15]. The rates of loss of CD56 IHC expression were significantly higher in malignant thyroid tumors, such as papillary thyroid carcinoma and follicular carcinoma than in FA and other benign lesions, such as follicular adenoma, benign follicular nodule, and Hashimoto's thyroiditis[16].

IHC markers initially were found in some studies to be a useful marker of malignancy, distinguishing malignant from benign conditions in thyroid diseases. However, these IHC markers of thyroid cancer, to our knowledge, have not been used in Mongolia. This study focuses on using these IHC markers in thyroid tumors in Mongolia. From experience gained in this study, it will become possible to effectively use these markers in practice going forward.

Materials and Methods

Study design and sampling

Fifty-three patients were diagnosed with thyroid neoplasms from September 2017 to November 2018 at National Cancer Center of Mongolia. Formalin-fixed paraffin-embedded tissue specimens from all patients were subjected to IHC staining manual methods. In all cases, IHC staining was performed using primary antibodies to CD56 (mouse, clone 123C3.D5, dilution 1:500, SIQMA-ALODRICH, USA), HBME-1 (mouse, clone HBME-1, dilution 1:100, SIQMA-ALODRICH, USA), Galectin-3 (mouse monoclonal, clone 9C4, dilution 1:100SIQMA-ALODRICH, USA), and CK19 (mouse, clone A53-B/A2.26,dilution 1:100, SIQMA-ALODRICH, USA) antigens on paraffin-embedded thyroid tumor sections.

Evaluation of immunohistochemical staining

Immunohistochemical staining was considered positive with cytoplasmatic with or without nuclear immunoreactivity for Galectin-3, membranous with or without cytoplasmatic immunoreactivity for immunostaining for cytokeratin-19 (CK19), and membranous with or without cytoplasmatic immunoreactivity for HBME-1, and membranous staining of follicular cells with CD56. The modified Allred scoring system was used to evaluate positivity, with staining intensity and distribution being scored separately. The staining intensity was scored as 0 (negative), 1 (weak), 2 (intermediate), or 3 (strong), and the distribution of positive-stained cells was semiquantitatively graded as 0 (negative), 1 (<1% positively stained cells), 2 (1-10% positively stained cells), 3 (11-33% positively stained cells), 4 (34-67% positively stained cells), or 5 (>67% positively stained cells). The total staining score was calculated as the sum of these two parameters. Total staining scores from 0 to 2 points were considered negative, while scores from 3 to 8 points were considered positive[17].

Statistical analysis

Descriptive and analytical methods were used. For comparison of variables, non-parametric tests (Fisher's Exact Test) were employed. Sensitivity, specificity, positive and negative predictive values were calculated for each of the markers. To compare markers, receiver operating curves (ROC) were constructed. Results were considered statistically significant when $p \leq .05$. The data analyses were performed using IBM SPSS Statistics version 20.

Ethical statements

This study was approved by Ethics Committee of Biomedical School, Mongolian National University of Medical Sciences on March 15, 2015.

Results

The thyroid tumors from 53 patients were studied. Eight (15.1%) were males, and forty-five (84.9%) were female. The mean age of patients was 45.11±13.9 years. Regarding the tumor size, 20 (37.7%) cases were less than 1 cm, and 33 (62.3%) cases were more than 1 cm. The median size of the tumors was 1.5 cm (range 0.3 to 7.00). The histopathological diagnosis of thyroid nodules was papillary thyroid carcinoma (Figure 1-A) in 40 (75.5%), follicular thyroid carcinoma in 3 (5.7%), follicular adenoma in 8 (15.1%), medullary thyroid carcinoma in 1 (1.9%), Hurthle cell carcinoma in 1 (1.9%), respectively. Nineteen (51.4%) had a pathologic T-stage of T1, 8 (21.6%) were stage T2, 7 (18.9%) were stage T3, and 3 (8.1%) were T4. In 25 cases, there was lymphovascular invasion (Table 1).

The immunohistochemical results for CD56, Galectin-3, HBME-1, and CK19 in the benign and malignant thyroid nodules were summarized in Table 2. Expressions of these four IHC markers were not relative to the tumor size, patients' age, or gender (p>.005). Out of 8 benign tumor cases, expression of CD56 was positive in 7 (87.5%), and CK19 positive in 1 (12.5%), but Galectin-3 and HBME-1 did not stain. Expression of CD56 was

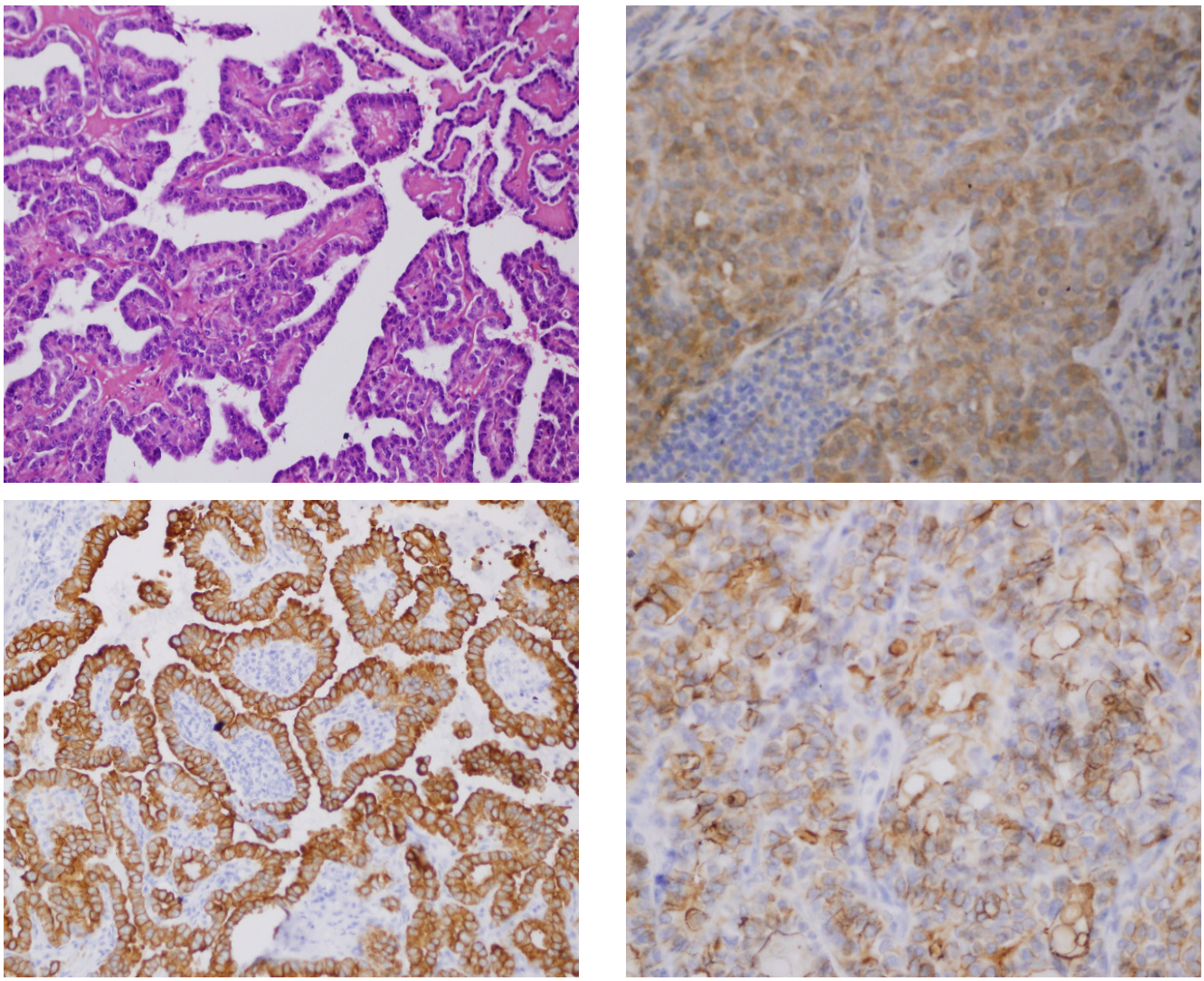

Figure 1. Classic papillary thyroid carcinoma histology using various stains in A-D (x40). A, H&E stain showing characteristic papillary architecture and nuclear features. B, Galectin-3 stain producing diffuse and strong membrane staining. C, Cytokeratin 19 stain showing diffuse and strong predominantly cytoplasmic staining. D, HBME-1 stain producing strong and diffuse cytoplasmic staining.

Table 1. Summary of clinicopathological findings

Parameters		All patients (N=53)
Gender	Male	8
	Female	45
Age (years)		
	Mean	45.11±13.9
	median	45
Diagnosis by Histology		
	Follicular adenoma	8
	Follicular carcinoma	3
	Papillary carcinoma	40
	Hurthle cell carcinoma	1
	Medullary carcinoma	1
pT-stage		
	T1	19
	T2	8
	T3	7
	T4	3
pN-stage		
	N0	7
	N1	21
	N2	2
	Nx	7
Lymphvascular invasion		
	Yes	25
	No	11
pM-stage		
	M0	6
	Mx	29
Tumor size (cm)		
	≤1	20
	>1	33

seen in 19 (42.2%), Galectin-3 was seen in 37 (82.2%), HBME-1 was seen in 33 (73.3%), and CK19 3 was seen in 6 (80%) of the malignant tumors. In Table 2, the difference in expression of Galectin-3 and CK19 was highly significantly different in malignant tumors compared to benign tumors (p=.000). Also, there were highly significant differences of HBME-1 marker seen in malignant tumors compared to benign tumors (p=.000), and significant differences in expression of CD56 between malignant and benign tumors (p=.024).

The among papillary carcinoma cases included 34 (85%) classic papillary variant of papillary thyroid carcinoma, 4 (10%) cases follicular variant of papillary carcinoma, 2 (5%) tall-cell of papillary carcinoma. Out of 40 cases of papillary thyroid carcinoma, Galectin-3 (Figure 1B) and CK19 (Figure 1C) showed positive in 36 (90%) cases while expressions of HBME1 (Figures 1D) was seen in 32 (80%) papillary thyroid carcinoma cases, respectively which 16 (40%) showed positive stained with CD56.

In Hurthle cell carcinoma, CK19, HBME1, and CD56 markers were not seen, but Galectin-3 was present. The CD56 marker was positive in only 1 of 3 follicular thyroid carcinoma cases, but the marker was positive in 7 out of 8 cases (87.5%) of follicular adenoma.

Contrasting papillary carcinoma and follicular adenoma, there were highly significant differences in expression of Galectin-3 in (90% vs. 0%, p=.000). There were significant differences between the expression of HBME-1 in papillary

Table 2. Expression of Galectin-3, CD56, HBME-1, and CK19 in benign and in malignant of thyroid tumors.

n	Total		Malignant		Benign		*p-value
	%	n	%	n	%		
CD56							.024
neg	27	50.9	26	57.8	1	12.5	
pos	26	49.1	19	42.2	7	87.5	
CK19							.0001
neg	16	30.2	9	20.0	7	87.5	
pos	37	69.8	36	80.0	1	12.5	
HBME-1							.0001
neg	20	37.7	12	26.7	8	100.0	
pos	33	62.3	33	73.3	0	0.0	
Galectin-3							.0001
neg	16	30.2	8	17.8	8	100.0	
pos	37	69.8	37	82.2	0	0.0	
Total	53	100.0	45	84.9	8	15.1	

*Fisher's Exact Test

carcinoma and in follicular adenoma (80% vs. 0%, p=.000). CK19 was more frequently positive in papillary carcinoma than in follicular adenoma (90% vs. 12.5%, p=.000). Also, CD56 was seen in a larger percentage of papillary carcinoma cases compared to follicular adenoma (40% vs. 87.5%, p=.02).

In papillary thyroid carcinoma, Galectin-3 had 100% sensitivity and 90% specificity while CK19 had a 87.5% sensitivity and 90% specificity using a total staining score of 2 as a cutoff point. At the same cut point, HBME-1 had a 100% sensitivity and 80% specificity, and the CD56 sensitivity was 87.5% with a specificity of 60%. The receiver operating characteristic of each stain for detecting papillary thyroid carcinoma are shown in Figure 2.

Discussion

Thyroid cancer is the most common endocrine malignancy, and more than 95% of thyroid carcinomas except medullary carcinoma originate from follicular epithelial cells. Out of all cases of follicular epithelial tumors, follicular adenoma, follicular carcinoma, and papillary carcinoma are most common. Medullary carcinomas that originate from parafollicular C cells, which are involved in the production of calcitonin, are rare, representing only about 3% of thyroid tumors[8]. HBME-1, Galectin-3, CK-19, CD56 have been commonly used markers for differential diagnosis of thyroid neoplasm. RET/PTC oncogene product immunostaining has been reported as a useful adjunct when used in combination with other antibodies, including CK19, galectin-3, and HBME-1 in the assessment of thyroid specimens and aspirates[8,18]. Among those markers, we aimed to determine the most suitable marker for papillary thyroid carcinoma, which is the most commonly diagnosed in thyroid cancer worldwide.

In this study, 84.9% (n=45) of patients were female with a mean age of 45.11±13.9 years. Moreover, we noticed that thyroid tumors tended to occur more often in female patients at a younger age and in middle age, which was similar to other studies.

In our study, we observed that the majority of cases were papillary thyroid carcinoma (75.5%, N=40), which was similar to other studies such as Dunderovic et al. (n=87). CK19, Galectin-3, and HBME-1 have all been shown to have higher expression in papillary thyroid carcinoma than follicular carcinoma in many studies[19].

Galectin-3 initially was found in some studies to be a useful marker of malignancy, distinguishing papillary carcinoma and follicular carcinomas from benign conditions[12]. Galectin-3 is normally expressed in macrophages, mast cells, Langerhans cells, and various malignant cells, including thyroid cells. It has

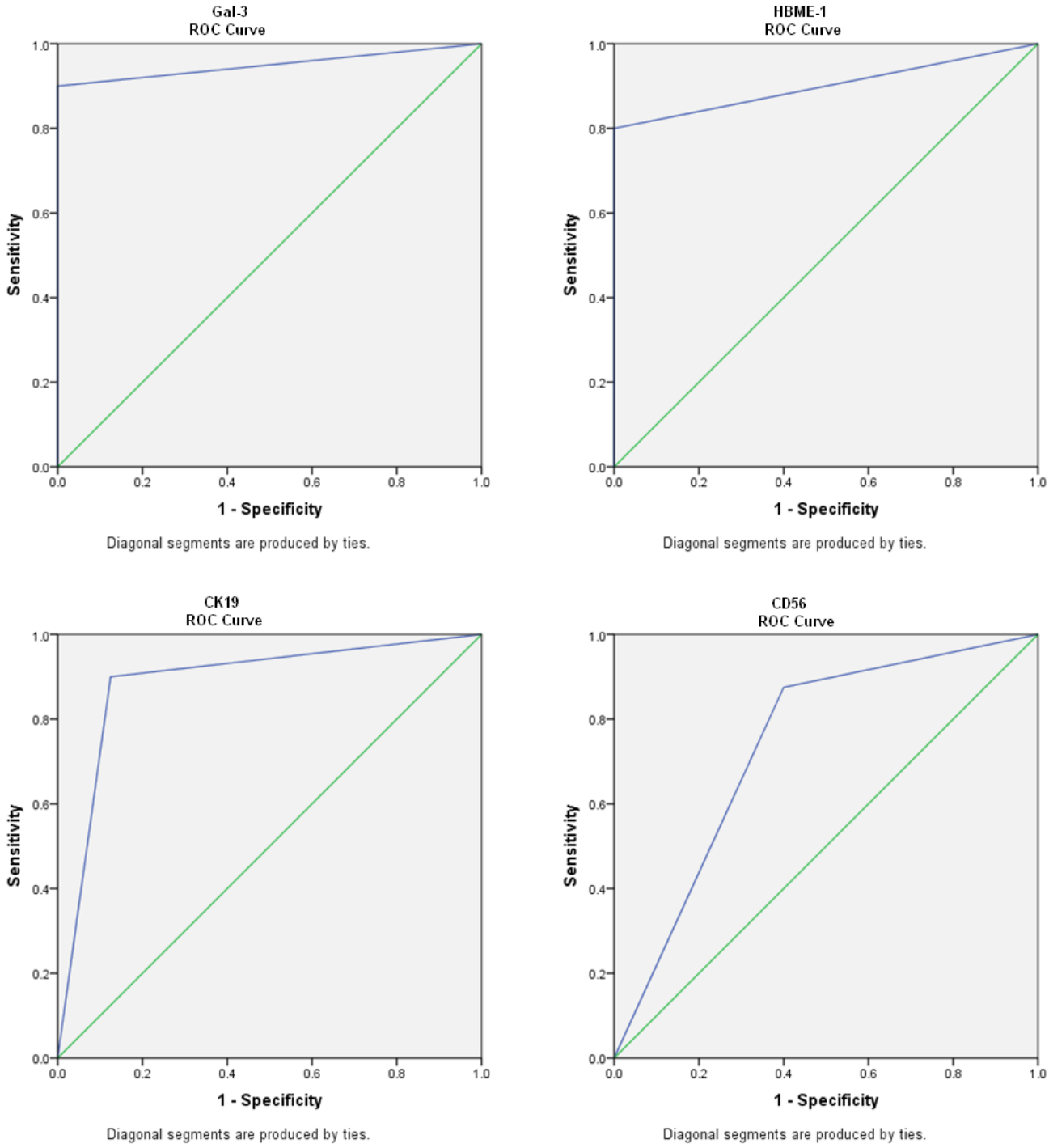

Figure 2. Receiver operating characteristic (ROC) curves for Galectin-3, HBME-1, CK19, and CD56 for papillary thyroid carcinoma.

been suggested that Galectin-3 could also play a role in the malignant transformation of thyroid cells and many studies have shown that papillary carcinoma, and especially classic papillary carcinoma, are characterized by strong, intense Galectin-3 expression[12]. In the study of Dunderovic et al., Galectin-3 was expressed significantly more in malignant tumors than in benign (88.5% vs. 35.4%, p=.000) as in our study in which it was expressed more commonly in malignant tumors (82.2% vs. 0%, p=.0001)[19].

Moreover, in Defining the value of CD56, CK19, Galectin

3 and HBME-1 in diagnosis of follicular cell-derived lesions of thyroid with systematic review of literature of Dunderovic et al., Galectin-3 was expressed in around 92% (n=80) of papillary thyroid carcinoma cases which was similar to the 90% (n=36) expression in our study[19,20]. However, we identified differences in expression of Galectin-3 in papillary carcinoma and in follicular adenoma (90% vs. 0%, p=.000) indicating that it may be possible to distinguish these two tumors with the help of Galectin-3. Also, Galectin-3 had had 100% sensitivity in detecting thyroid tumor cells which was the highest in sensitivity out of all the markers.

Papillary carcinomas have been shown to express strong and diffuse immunoreactivity for CK7, CK18, and CK19 in 80% to 100% of cases[21,22]. CK19 is a low molecular weight cytokeratin, present in a wide range of normal and neoplastic tissues. Several authors emphasize the importance of the distribution and intensity of CK19 staining as the most critical aspects of accurate interpretation. Normal thyroid follicular epithelium is often negative, although focal staining for CK19 is usually identified in the compressed thyroid parenchyma surrounding nodules and in follicular cells within lymphocytic thyroiditis[8].

In our study, expression of CK19 was seen in 80% (n=45) of malignant tumor cases and 12.5% (n=8) of benign tumor cases while CK19 expression was present in 75.4% (n=92) of malignant tumors, generally with high intensity and wide distribution, and in 29.1% (n=23) of benign lesions in Dunderovic et al.[19]. In other studies, such as the study of Song et al., CK19 was shown to have high expressions in papillary thyroid carcinoma cases[20]. This was comparable to our study in which 90% (n=36) positive staining in papillary thyroid carcinoma. As a result, we identified differences in expression of CK19 in papillary carcinoma and in follicular adenoma (90%% vs. 12.5%, p=.000) and sensitivity level of CK19 expression in thyroid tumor which was quite high (87.5%).

HBME-1 is a monoclonal antibody that recognizes an unknown antigen in the microvilli of mesothelioma cells, normal tracheal epithelium, and adenocarcinoma of the lung, pancreas, and breast. HBME-1 is abnormally expressed in thyroid cancer, seen in cytoplasm with membrane accentuation, and is usually negative in normal follicular cells. Interestingly, membranous and apical-colloidal immunoreactivity for HBME-1 has been reported in follicular carcinomas with RAS mutations, either minimally or widely invasive. In our study, expression of HBME-1 was seen in 73.3% (n=33) of malignant tumor cases. It was also seen in 71.3% (n=87) of malignant tumor cases in the study of Dunderovic et al., a result very similar to ours[19].

In our study, out of 40 cases of papillary thyroid carcinoma, expression of HBME1 was seen in 80% (n=32) papillary thyroid carcinoma cases while it was seen in 88% of papillary thyroid carcinoma cases in the work of Scognamiglio et al. As a result, we identified differences in expression of HBME-1 in papillary carcinoma and in follicular adenoma (80% vs. 0%, p=.000) which opened the possibility of distinguishing between these two tumors using this stain. Also, the specificity (80%) and sensitivity (100%) of HBME-1 was quite high for thyroid tumor cells.

CD56 is a cell surface glycoprotein which is usually expressed in normal thyroid follicular cells and is preserved in almost all benign thyroid tumors. Decreased expression of CD56 has mostly been found in malignant thyroid nodules, especially in papillary thyroid carcinoma, thus its diagnostic utility in comparing thyroid tumors. In other studies, loss of CD56 expression was correlated with metastatic potential and poor prognosis in some malignant tumors. In a study by Song et al. differences of CD56 expression were significant between papillary carcinoma and follicular adenoma (p = .000) which was not similar to our study[12]. This discrepancy may be explained by the fact that we collected a limited number of benign thyroid lesions, especially follicular adenoma.

Our study was limited by the relatively few numbers of cases of thyroid tumor, especially follicular adenomas. However, this did not have a substantial effect on our results. In the long run, we will define gene mutations of BRAF, RET/PTC, RAS and PAX8/PPARgin these thyroid tumor cases. Furthermore, the correlation between gene mutations and expressions of the markers will be studied.

In conclusion, our results suggest Galectin-3 and HBME-1 are the most sensitive diagnostic markers for papillary carcinoma differentiating from follicular neoplasm. The combination of Galectin-3, CK-19, HBME-1, and CD56 markers had a significant impact on the differential diagnosis of thyroid neoplasm.

Conflict of Interest

The authors have no potential conflicts of interest to disclose.

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