REVIEW ARTICLE

The Diagnostic Value of Various Immunohistochemical Biomarkers in the Detection of Papillary and Follicular Thyroid Cancers: A Systematic Review and Meta-analysis

Mitra Heidarpour[1], MD; Yalda Heshmaty[1], MD; Alireza Rahimi[2], PhD; Awaz Feizi[3], PhD; Reza Rakhshan[1], MD; Maryam Ghasemi[4], MD

[1]Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Department of Management and Health Information Technology, Isfahan, Iran
[3]Department of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Student Research Committee, University of Medical Sciences, Shahrekord, Iran

Correspondence:
Mitra Heidarpour, MD;
Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
Email: mitraheidarpour@gmail.com
Received: 21 January 2024
Revised: 23 February 2024
Accepted: 25 March 2024

Abstract

Background: Thyroid neoplasia is the most common endocrine malignancy worldwide. Fine-needle aspiration biopsy of thyroid nodules has a low sensitivity in distinguishing between benign and malignant lesions. Evaluation of the rate of expression and diagnostic value of immunohistochemical biomarkers in differentiating between benign and malignant thyroid lesions and different types of malignant lesions is the main purpose of this study.

Methods: Sixty articles were reviewed in this systematic review and meta-analysis study. The rate of detection of various immunohistochemistry (IHC) biomarkers in several thyroid lesions was examined by meta-analysis. Specificity, sensitivity, positive and negative likelihood ratios, and confidence intervals (95% CI) were calculated for each marker. The accuracy of each test was evaluated by calculating the diagnostic odds ratio (DOR). ROC (receiver operating characteristic) analysis was performed for three markers.

Results: Sensitivity and specificity of CK-19, Gal-3, and carcinoembryonic antigen (CEA) for detection of thyroid malignancies were 81% and 73%, 82% and 81%, and 77% and 83 %, respectively. The combination of these three markers showed the sensitivity of 85%, specificity of 97%, and diagnostic odds ratio of 95.1. Additionally, uPAR, Sialyl Lewis X, MIB-1, and Hector Battifora mesothelial-1. (HBME-1) can effectively differentiate the follicular variant of papillary thyroid carcinoma (FVPTC) from follicular thyroid carcinoma (FTC) as they are significantly more common in FVPTCs ($P<0.05$).

Conclusion: We showed that CK-19, Gal-3, and CEA had an important and statistically significant role in differentiating between benign and malignant thyroid lesions. In addition, according to our results, urokinase-type plasminogen activator receptor (uPAR), Sialyl Lewis X, MIB-1, and HBME-1 can effectively differentiate FVPTC from FTC with acceptable sensitivity and specificity.

Please cite this article as: Heidarpour M, Heshmaty Y, Rahimi AR, Feizi A, Rakhshan R, Ghasemi M. The Diagnostic Value of Various Immunohistochemical Biomarkers in the Detection of Papillary and Follicular Thyroid Cancers: A Systematic Review and Meta-analysis. J Health Sci Surveillance Sys. 2024;12(2):125-133.

Keywords: Thyroid neoplasms, Papillary thyroid cancers, Follicular thyroid carcinomas

Introduction

Thyroid neoplasia is the most common endocrine malignancy worldwide. Based on global cancer statistics, 52,890 cases of thyroid cancer were discovered in the US in 2020, with 2,180 of them ending in death.[1]

A cost-effective and easily accessible test for diagnosing malignancy is fine-needle aspiration biopsy (FNAB) of thyroid nodules. Based on the cytological evaluation of FNAB samples, nodules are categorized as benign (70%), malignant (5-10%), indeterminate (10-20%) or not otherwise specified (NOS) (10-15%).[2-4] The most prominent issue with FNAB is that it has a low sensitivity in distinguishing between benign and malignant (e.g. follicular adenoma versus carcinoma) lesions.[5]

Thyroid tumors are heterogeneous and new pathological entities are constantly emerging. Establishing a correct pathological diagnosis and differentiating between well-differentiated tumors such as follicular carcinoma and follicular variant papillary carcinoma can be difficult, even for experienced thyroid pathologists. Papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC) are the two main types of thyroid cancer. The former is mainly composed of follicles and the latter is characterized by solid areas and clear, papillary nuclei.

Many studies have shown that using immunohistochemistry (IHC) to detect different thyroid biomarkers can be promising in differentiating thyroid lesions. Galectin-3, cytokeratin 19, P53, monoclonal carcinoembryonic antigen (CEA), FOXE1, and e-cadherin are the most common markers employed for immunohistochemical evaluation of thyroid lesions.[6, 7] However, the application of these biomarkers in the diagnosis of thyroid malignancies is still controversial, as their specificity and sensitivity for differentiating between follicular and papillary lesions have not yet been determined.[8]

Evaluation of the rate of expression and diagnostic value of galectin-3, cytokeratin 19, P53, monoclonal carcinoembryonic antigen (CEA), FOXE1, and other biomarkers in the benign and malignant types of thyroid lesions is the main purpose of this systematic review and meta-analysis study. The second aim is to determine the value of immunohistochemistry markers in differentiating between papillary and follicular thyroid cancers.

Methods

Bibliographic databases including PubMed, MEDLINE, and Cochrane Library electronic databases were searched for related articles. Articles published between January 2000 and January 2022 were included. To avoid publication bias, we used an extensive search strategy with the subsequent keywords: *(CEA and thyroid)) OR ((CK-19 and thyroid) OR (galectin-3 and thyroid) OR (FOXE1 and thyroid) OR (P53 and thyroid) OR (E-cadherin and thyroid) OR (GATA-3 and thyroid) OR (HHEX and thyroid) OR (beta-catenin and thyroid) OR (uPAR and thyroid) OR (sialyl Lewis X and thyroid) OR (MIB-1 and thyroid).* Other pertinent studies were identified by analyzing the references of previously retrieved articles.

All the original articles with participants over 18 years of age were included. Any study without case or control groups or fewer than 12 patients in each group was excluded. Studies evaluating non-malignant thyroid lesions, such as goiter, adenoma, thyroiditis, or hyperplasic nodules were excluded. Case reports and studies that investigated any marker other than those mentioned above or used non-immunocytochemistry diagnostic techniques were also excluded.

Statistical Analysis

The Comprehensive Meta-Analysis software package (Biostat, Englewood, NJ, USA) was used for data analysis. The rate of detection of various IHC biomarkers in several thyroid lesions was examined by meta-analysis. Subgroup analysis was based on lesion variants and Q and I^2 statistics, and P values were calculated to check heterogeneity between studies. A sensitivity analysis of heterogeneity of eligible studies was also performed. A meta-regression test was used to assess the difference between the subgroups, and P-values were presented. The fail-safe N and trim-fill tests were performed only if a substantial publication bias was found. $P<0.01$ was considered as the significance level.

Specificity, sensitivity, positive and negative likelihood ratios, and confidence intervals (95% CI) were calculated. Forest plots of the most associated results were also drawn. The accuracy of the test was evaluated by calculating the diagnostic odds ratio (DOR).

ROC (receiver operating characteristic) analysis was performed and areas under the summary ROC curves were measured.

PICO

Problem: The most important immunohistochemical biomarkers in papillary and follicular thyroid cancers

Patients: Patients with papillary and follicular thyroid cancer.

Intervention or exposure: no intervention

Comparison: Comparison of the IHC markers in two common thyroid cancers

Outcome: the most valuable biomarkers in detecting the two most important and common thyroid cancers.

Results

The diagnostic accuracy of IHC markers including CEA, CK-19, galectin-3, FOXE1, P53, E-cadherin, GATA-3, HHEX, and beta-catenin and their value in distinguishing between thyroid carcinomas and benign thyroid lesions are described separately below.

Immunohistochemistry Technique

Sixty articles and 5632 patients were included in this study. The number of included studies and patients and true and false negative and positive test results for each IHC marker are displayed in Table 1. Sensitivity, specificity, likelihood ratios, and respective heterogeneity coefficients for each IHC marker are shown in Tables 2 and 3. The diagnostic odds ratio (DOR) for each IHC marker was calculated directly using sensitivity and specificity values (Table 4). The diagnostic odds ratio represents the overall diagnostic power of each test (a high DOR indicates that the test diagnosed most patients correctly). Forest plots of sensitivity, specificity, and positive and negative likelihood ratios of the combination of CK-19, Gal-3, and CEA in the diagnosis of malignant thyroid lesions are shown in Figures 1 and 2.

Assessing Heterogeneity

The main factor contributing to heterogeneity

Table 1: The number of studies, patients, and test results for different immunohistochemistry markers

IHC analysis	Patients	Studies	TP	FP	FN	TN
CEA[51-54]	552	19	265 (48.0%)	72 (13.04%)	75 (13.58%)	140 (25.36%)
CK-19[24,25,36]	3712	32	1726 (46.4%)	409 (11.1%)	167 (4.49%)	1410 (38.0%)
galectin-3[10,16,17-22,50-59]	6023	49	2580 (42.83%)	460 (7.63%)	995 (16.52%)	1988 (33.0%)
FOXE1[31-35]	258	12	135 (52.32%)	6 (2.32%)	24 (9.30%)	93 (36.04%)
P53[44-47]	3600	6	1823 (50.63%)	227 (6.30%)	506 (14.05%)	1044 (29.0%)
E-cadherin[64-67]	335	9	178 (53.13%)	14 (4.17%)	23 (6.86%)	120 (35.82%)
GATA-3[48,49]	151	3	81 (53.64%)	10 (6.62%)	8 (5.29%)	52 (34.43%)
HHEX[68-70]	233	4	115 (49.35%)	15 (6.43%)	30 (12.87%)	73 (31.33%)
beta-catenin[71,72]	146	3	78 (53.42%)	6 (4.10%)	15 (10.27%)	47 (32.19%)

Table 2: Sensitivity and specificity of immunohistochemistry markers

IHC analysis	Sensitivity	Q	P	Specificity	Q	P
CEA[51-54]	0.78 (0.75-0.80)	186.03	<0.00001	0.72 (0.70-0.74)	246.96	<0.00001
CK-19[24,25,36]	0.79 (0.80-0.83)	332.63	<0.00001	0.82 (0.79-0.84)	635.26	<0.00001
galectin-3[10,16,17-22,50-59]	0.75 (0.74-0.79)	305.38	<0.00001	0.88 (0.82-0.96)	553.32	<0.00001
FOXE1[31-35]	0.73 (0.74-0.76)	265.73	0.3652	0.89 (0.88-0.93)	444.61	<0.00001
P53[44-47]	0.74 (0.73-0.77)	153.48	0.6638	0.90 (0.88-0.96)	6.30	<0.00001
E-cadherin[64-67]	0.69 (0.70-0.73)	0.36	0.7820	0.92 (0.90-0.93)	7.18	<0.00001
GATA-3[48,49]	0.67 (0.65-0.69)	0.86	0.9963	0.89 (0.89-0.93)	6.39	<0.00001
HHEX[68-70]	233	0.77	0.2201	0.96 (0.89-0.97)	6.97	0.0035
beta-catenin[71,72]	146	66.37	0.9663	0.92 (0.83-0.93)	5.66	0.00365
galectin-3 and CK-19[23,23-30,37-43]	0.82 (0.80-0.86)	68.15	<0.00001	0.88 (0.82-0.96)	6.56	0.0563
galectin-3, CK-19, and CEA[60-63]	0.80 (0.81-0.83)	0.63	<0.00001	0.98 (0.90-0.99)	6.68	0.03663

Table 3: Positive likelihood ratio (Positive LR) and negative likelihood ratio (Negative LR) of immunohistochemistry markers

IHC analysis	Positive LR (95% CI)	Q	P	Negative LR (95% CI)	Q	P value
CEA[51-54]	3.88 (3.12-3.93)	332.63	<0.00001	0.72 (0.70-0.74)	246.96	<0.00001
CK-19[24,25,36]	4.42 (3.12-5.86)	305.38	<0.00001	0.82 (0.79-0.84)	635.26	<0.00001
galectin-3[10,16,17-22,50-59]	6.65 (5.02-7.63)	265.73	<0.00001	0.88 (0.82-0.96)	553.32	<0.00001
FOXE1[31-35]	15.32 (14.22-16.63)	153.48	0.0323	0.89 (0.88-0.93)	444.61	<0.00001
P53[44-47]	4.42 (2.09-6.91)	366.32	0.0098	0.90 (0.88-0.96)	6.30	<0.00001
E-cadherin[64-67]	7.38 (5.39-9.32)	9.39	0.00145	0.92 (0.90-0.93)	7.18	<0.00001
GATA-3[48,49]	2.87 (2.36-4.59)	8.26	00.00236	0.89 (0.89-0.93)	6.39	<0.00001
HHEX[68-70]	7.78 (5.14-9.36)	7.19	0.02169	0.96 (0.89-0.97)	6.97	0.0035
beta-catenin[71,72]	4.43 (3.96-6.38)	7.39	<0.00001	0.92 (0.83-0.93)	5.66	0.00365
galectin-3 and CK-19[23,24-30,37-43]	8.75 (7.21-9.63)	10.83	<0.00001	0.88 (0.82-0.96)	6.56	0.0563
galectin-3, CK-19, and CEA[60-63]	16.63 (15.02-17.22)	2.96	<0.00001	0.98 (0.90-0.99)	6.68	0.03663

Table 4: Diagnostic odds ratio (DOR) of immunohistochemistry markers

IHC analysis	DOR (95% CI)	Q	P value
CEA[51-54]	15.53 (10.02-20.65)	16.61	<0.00001
CK-19[24,25,36]	26.42 (14.46-39.68)	22.28	<0.00001
galectin-3[10,16,17-22,50-59]	45.73 (23.87-53.17)	43.92	<0.00001
FOXE1[31-35]	124.83 (7.73-1083.91)	507.92	0.6032
P53[44-47]	76.37 (51.19-108.83)	81.08	0.4233
E-cadherin[64-67]	23.36 (10.77-90.75)	43.91	0.8375
GATA-3[48,49]	88.19 (60.73-110.68)	81.76	0.7083
HHEX[68-70]	101.05 (62.85-209.62)	134.50	<0.00001
beta-catenin[71,72]	21.94 (2.88-193.94)	129.13	0.4420
galectin-3 and CK-19[23,23-30,37-43]	15.37 (6.86-38.19)	32.27	0.09533
galectin-3, CK-19 and CEA[60-63]	95.06 (25.19-403.80)	229.93	0.77532

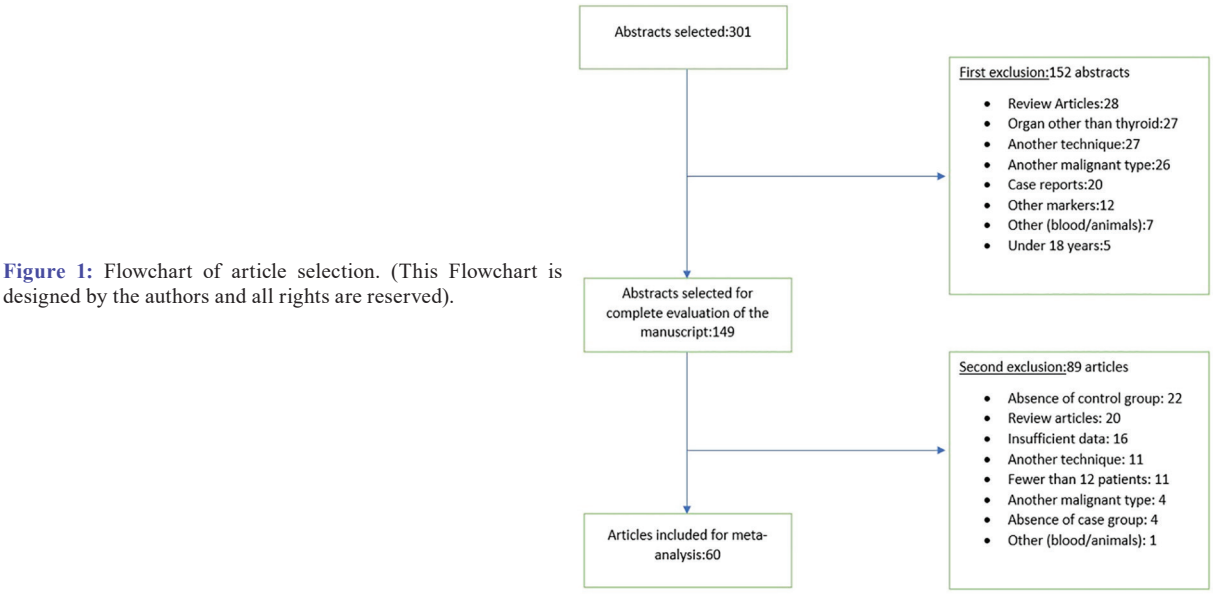

Figure 1: Flowchart of article selection. (This Flowchart is designed by the authors and all rights are reserved).

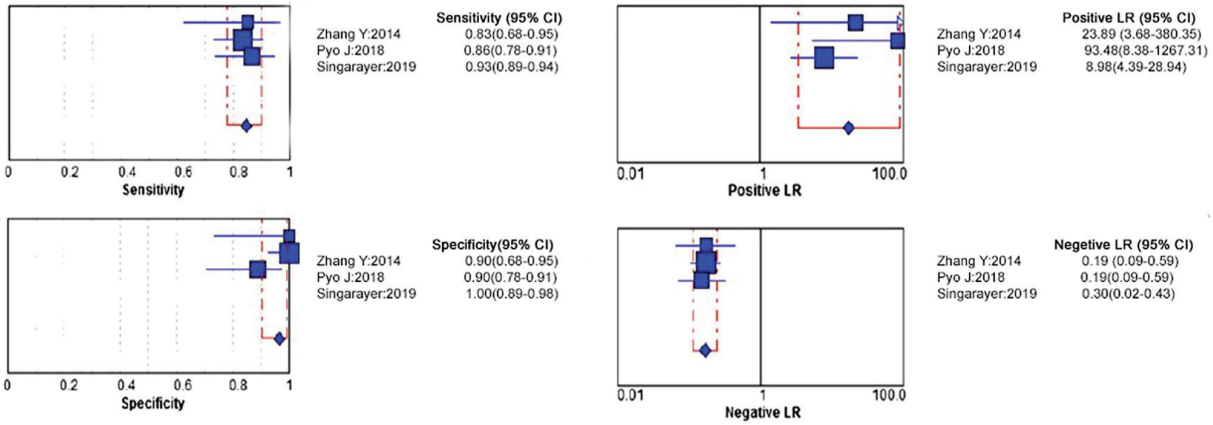

Figure 2: Forest-plot graph for sensitivity, specificity, positive and negative likelihood ratio of immunohistochemistry expression of the positive combination of CK-19, Gal-3, and CEA in the diagnosis of malignant thyroid lesions (This image is designed by the authors and all rights are reserved).

among the studies was using a combination of IHC markers for diagnosis. As a result, when evaluated separately, none of the IHC biomarkers was able to correctly distinguish between benign and well-differentiated papilloma and follicular carcinomas. Therefore, the existence of the following possible confounding factors was examined among the studies: 1. the criteria selected to define a marker as "positive" and 2. the inclusion of Hurthle cells in the evaluation.[9]

A review of the included articles indicated that, regardless of other criteria, some studies had considered the presence of oncocytic pattern (Hurthle cells) in the cytological examinations to represent a higher likelihood of malignancy (Bethesda IV).[10] Moreover, in some studies, a positive test result was defined as the expression of the biomarker in more than 5% of the cells. In contrast, other studies regarded more than 10%, 25%, or even 50% expression as positive.[11-18]

Accordingly, we re-categorized the results of the included studies. All samples that had less than 25% biomarker expression were defined as weak or negative. As a result, the previously noted heterogeneity among the studies was eliminated.

SROC curves were plotted for immunohistochemistry expression of three markers (CK-19, Gal-3, and CEA) (Figure 3). It should be noted that we conducted a new separate analysis for these three markers after the removal of confounding variables; therefore, unbalanced heterogeneity was present. Because published articles and not the original data of the studies were used in our meta-analysis, the mentioned criteria were not available for evaluation in some articles, which were, therefore, excluded.

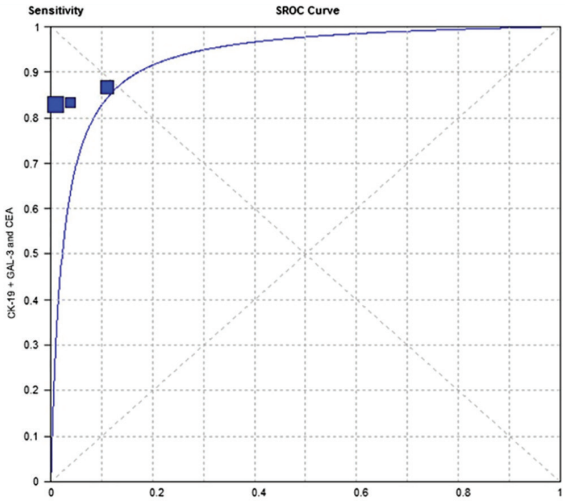

Figure 3: SROC curve for immunohistochemistry expression of three markers (CK-19, Gal-3 and CEA) (This image is designed by the authors and all rights are reserved).

Differentiation between FVPTC and FTC

In the initial search, 209 manuscripts were found on this topic. In accordance with the inclusion and exclusion criteria, 140 manuscripts were excluded. The full texts of the 69 remaining articles were analyzed, and 49 publications were excluded. Twenty publications were included in the final review. The evaluated IHC biomarkers in these studies included uPAR, sialyl Lewis X, MIB-1, HBME-1, Ret, CK-19, and S100A4. Analysis of the ability of these biomarkers to distinguish between FVPTC and FTC is shown in Table 5. The reported OR is the odds of FVPTC staining with the particular biomarker compared to FTC. OR<1 means that FVPTC will stain more than FTC for that specific marker. Some OR results were equal to zero or infinite due to mathematical reasons. Seven of the evaluated biomarkers had statistically significant results (Table 5), which means they were able to differentiate between the two thyroid lesions.

Urokinase-type Plasminogen Activator Receptor (uPAR)

Ivanova et al. investigated the diagnostic value of urokinase-type plasminogen activator receptor (uPAR) which is a GPI-anchored cell membrane receptor composed of three homologous domains.[19] Their results revealed that all FVPTC were uPAR positive, whereas 87% of FTC were negative for this immunostaining (P<0.001). The sensitivity and specificity of uPAR for differentiating between FVPTC and FTC were 89% and 90.74%, respectively. The computed OR (for FTC compared to FVPTC) was 0.001 (95% CI 0.0001-0.019).

Sialyl Lewis X

The tetrasaccharide carbohydrate Sialyl Lewis X, also called cluster of differentiation 15s or stage-specific embryonic antigen 1, is attached to O-glycans on the surface of cells. It has a critical role in cellular recognition processes. Ivanova et al. investigated the expression of Sialyl Lewis X in 19 FTC and 20 FVPTC cases. According to the results, Sialyl Lewis X was expressed in all FVPTCs but only in 16% of FTCs (P<0.01). The calculated OR for FTC compared to FVPTC for Sialyl lewis X staining was 0.0076 (95% CI 0.0004-0.15). OR for Sialyl Lewis X immunostaining for FVPTC compared to FTC was 131.[19-21]

MIB-1

The rate of expression for Ki-67 (MIB-1 clone which binds to the Ki-67 antigen and leads to DNA synthesis) was 96% (26/27) in FTCs. In contrast, it was not expressed in 83% (25/30) of FVPTCs. The sensitivity and specificity of this biomarker for differentiating FTC from FVPTC were 97% and 83%, respectively. The calculated OR was 130 (95% CI 14.2-1192.3).[22-25]

HBME-1

HBME1 is a monoclonal antibody which reacts with the microvillous surface of the mesothelial cells.

Table 5: The ability of IHC biomarkers to differentiate between FVPTC and FTC

Biomarker	Sensitivity	Specificity	Calculate OR (FTC>FVPTC)	Calculated 95% CI	P value
uPAR	100 %	90.74 %	0.001 (FVPTC>FTC 1000)	0.0001 to 0.019	<0.001
Sialyl Lewis X	96 %	91 %	0.0076 (FVPTC>FTC 131)	0.0004 to 0.15	<0.01
MIB-1	97%	83 %	130	14.2 to 1192.3	<0.01
HBME-1	-	-	0.0054 (FVPTC>FTC 200)	-	<0.002
RET	89%	83%	0.0054 (FTC>FVPTC 31)	6.28 to 97.1	<0.003
CK-19	-	-	Different strain pattern	-	<0.05
S1004A	-	-	Different strain pattern	-	<0.05

It was initially used to examine mesothelioma, but it has recently been used for detection of malignant thyroid disease. De Matos et al. assessed the ability of this biomarker to differentiate between FVPTC and FTC. According to their analysis, no FTCs expressed HBME-1, whereas 12/14 (85.7%) of FVPTCs highly expressed this marker.[17-26]

RET

RET is a tumor suppressor gene involved in the pathogenesis of colorectal cancer and is not expressed in normal thyroid cells. Results of the analysis done by Shin et al. showed that all 21 (100%) FTCs stained positively for RET, whereas 88% (23/26) of FVPTCs stained negative for this marker.[27]

CK-19

Cytokeratin 19 (CK-19) is a keratin mainly expressed in the gastroenteropancreatic and hepatobiliary tracts. CK-19 IHC has been used in differentiating papillary carcinomas. Jain et al. surveyed the IHC expression of CK-19 in FVPTCs and FTCs. They demonstrated that 94% of FVPTCs had apical and/or polar CK-19 staining. In contrast, 43% of FTCs showed diffuse staining for this marker ($P<0.005$). Sensitivity and specificity of CK-19 for differentiating between FVPTC and FTC were 95% and 100%, respectively. OR was calculated zero due to the similar numbers of slides with positive CK-19 immunostaining in both groups.[28]

S1004A

S100A4 is a Ca-binding protein which was previously used to detect metastatic tumors. Ito et al. investigated the expression of S100A4 in 18 FVPTC and 16 FTC cases. Their results showed that the intensity of S1004A expression in FTCs was weaker compared to FVPTCs ($P<0.05$). The marker demonstrated a membranous pattern for FTCs in contrast to predominantly cytoplasmic staining in FVPTCs ($P<0.05$). OR was zero due to similar numbers of slides with positive immunostaining in both groups.[29]

Discussion

Accurate diagnosis of thyroid lesions and differentiation between malignant and benign lesions can be challenging, even for experienced pathologists.[30-32] Accordingly, the development of a non-invasive and accurate diagnostic test to differentiate between various thyroid lesions has been one of the main focuses of recent research.[33] However, despite using genomic classifiers, this aim has not yet been achieved. Therefore, many researchers have examined the diagnostic capability of other markers, including IHC markers, in the diagnosis of thyroid lesions.[26, 34, 35]

Cytokeratin-19 (CK-19) expression is diffuse in papillary carcinoma and heterogeneous in follicular carcinoma and follicular adenoma, but low in benign lesions. Galectin-3 is proposed to have a critical role in the pathogenesis of highly differentiated papillary carcinoma and is, therefore, commonly used to distinguish between different thyroid lesions.[36, 37]

Carcinoembryonic antigen (CEA) has been demonstrated to originate from malignant thyroid follicles.38 Accordingly, these markers can be used for pathological differentiation of thyroid lesions. It has been suggested that using a panel of these markers might provide better specificity and positive and negative predictive values and, therefore, greater diagnostic accuracy than a single immunomarker. The present study provided an estimate of the accuracy of these markers in clinical practice. Various review articles have been published on this topic, but this study is one of the first investigation done to evaluate cumulative data.[29-31]

Using the combination of galectin-3, CK-19, and CEA in IHC evaluation was revealed to be the most precise test in differentiating between benign lesions and high-grade thyroid carcinoma with a global accuracy of more than 90% based on the SROC curve. Nevertheless, these results need to be interpreted carefully. It is prudent to use a follow-up confirmatory test because all IHC markers can produce false positive or negative results. This leads to prevention of misdiagnosis and unnecessary surgical procedures and the resulting morbidity and mortality.[3]

The main factors contributing to heterogeneity among the reviewed studies were use of a combination of markers, exclusion of hurthle cells, and use of different criteria for defining positive results. It is possible that a heterogeneous outcome is also caused by different technical methods used in the IHC assays, such as specimen fixation, use of monoclonal or polyclonal antibodies, and methods for non-biotin-based detection. However, these differences were not evaluated in this study. Future reviews should standardize these parameters to enhance the accuracy evaluation of the IHC method and achieve uniformity among the studies.

The follicular variant of papillary carcinoma (FVPTC) is characterized by follicular growth patterns and tumor cells with nuclear features of papillary carcinoma.31 On rare occasions, these lesions may exhibit focal or multifocal nuclear features instead of the diffuse distribution usually seen in papillary carcinoma. Therefore, these lesions may be misdiagnosed as benign follicular nodule or malignant follicular carcinoma.28

Differentiating FVPTC from FTC persists as a diagnostic dilemma.[30] In our study, numerous IHC

biomarkers which were frequently used across laboratories to distinguish FVPTC from FTC were examined.[32, 33, 39, 40] There is not an IHC marker with consistently reproducible results that can reliably distinguish the two types of tumors. However, this analysis demonstrated some IHC biomarkers that may help this differentiation. These markers need to be validated in more extensive international collaborative studies.

Conclusion

We surveyed several immunohistochemistry markers that had previously been shown as important identifiers for diagnosing thyroid malignancies. We showed that CK-19, Gal-3, and CEA had an important and statistically significant role in differentiating between benign and malignant thyroid lesions before and after thyroid surgery. In addition, according to our results, uPAR, Sialyl Lewis X, MIB-1, and HBME-1 can effectively differentiate FVPTC from FTC with acceptable sensitivity and specificity. Continued search for other diagnostic molecular markers is recommended as our findings demonstrated that all of these markers produced some false negative and false positive results.

Authors' Contribution

All the authors made significant contributions to this study. In addition, all authors have agreed to take responsibility for the accuracy and integrity of the work as a whole.

Acknowledgement

The authors would like to express their sincere gratitude to all authors and co-authors for their invaluable contributions and support throughout this research. We extend our appreciation to the Isfahan and Shahrekord Universities of Medical Sciences for providing the necessary resources and facilities for this research.

Ethical Issues

This systematic review and meta-analysis was conducted in accordance with the World Medical Association Declaration of Helsinki. This study was performed after obtaining the approval of the ethics committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1400.107). It is a part of obstetrics and gynecology residency thesis of Yalda Heshmati at this university. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/support

This study received no grants or fundings.
Conflict of Interest: None declared.

References

1 Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010 Sep-Oct;60 (5):277-300. Erratum in: CA Cancer J Clin. 2011;61 (2):133-4. doi: 10.3322/caac.20073. PMID: 20610543.

2 Tuchscherer M, Otten W, Kanitz E, Gräbner M, Tuchscherer A, Bellmann O, Rehfeldt C, Metges CC. Effects of inadequate maternal dietary protein:carbohydrate ratios during pregnancy on offspring immunity in pigs. BMC Vet Res. 2012; 8:232. doi: 10.1186/1746-6148-8-232. PMID: 23190629; PMCID: PMC3527219.

3 Peccin S, de Castsro JA, Furlanetto TW, Furtado AP, Brasil BA, Czepielewski MA. Ultrasonography: is it useful in the diagnosis of cancer in thyroid nodules? J Endocrinol Invest. 2002;25 (1):39-43. doi: 10.1007/BF03343959. PMID: 11885575.

4 Tomimori EK, Bisi H, Medeiros-Neto G, Camargo RYA de. Avaliação ultra-sonográfica dos nódulos tireóideos: comparação com exame citológico e histopatológico. Arq Bras Endocrinol Metab [Internet]. 2004;48 (1):105–13. doi: 10.1590/S0004-27302004000100012.

5 Utiger RD. The multiplicity of thyroid nodules and carcinomas. N Engl J Med. 2005 Jun 9;352 (23):2376-8. doi: 10.1056/NEJMp058061. PMID: 15944422.

6 Schlumberger MJ. Papillary and follicular thyroid carcinoma. N Engl J Med. 1998; 338 (5):297-306. doi: 10.1056/NEJM199801293380506. PMID: 9445411.

7 LiVolsi VA, Baloch ZW. Follicular neoplasms of the thyroid: view, biases, and experiences. Adv Anat Pathol. 2004; 11 (6):279-87. doi: 10.1097/01.pap.0000138143.34505.02. PMID: 15505528.

8 Zeiger MA, Dackiw AP. Follicular thyroid lesions, elements that affect both diagnosis and prognosis. J Surg Oncol. 2005; 89 (3):108-13. doi: 10.1002/jso.20186. PMID: 15719377.

9 Barroeta JE, Baloch ZW, Lal P, Pasha TL, Zhang PJ, LiVolsi VA. Diagnostic value of differential expression of CK19, Galectin-3, HBME-1, ERK, RET, and p16 in benign and malignant follicular-derived lesions of the thyroid: an immunohistochemical tissue microarray analysis. Endocr Pathol. 2006; 17 (3):225-34. doi: 10.1385/ep:17:3:225. PMID: 17308359.

10 Saussez S, Glinoer D, Chantrain G, Pattou F, Carnaille B, André S, Gabius HJ, Laurent G. Serum galectin-1 and galectin-3 levels in benign and malignant nodular thyroid disease. Thyroid. 2008; 18 (7):705-12. doi: 10.1089/thy.2007.0361. PMID: 18630998.

11 Yoshii T, Inohara H, Takenaka Y, Honjo Y, Akahani S, Nomura T, Raz A, Kubo T. Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. Int J Oncol. 2001; 18 (4):787-92. doi: 10.3892/ijo.18.4.787. PMID: 11251175.

12 Baloch ZW, Abraham S, Roberts S, LiVolsi VA. Differential expression of cytokeratins in follicular variant of papillary carcinoma: an immunohistochemical

study and its diagnostic utility. Hum Pathol. 1999; 30 (10):1166-71. doi: 10.1016/s0046-8177 (99)90033-3. PMID: 10534163.

13 Kato MA, Fahey TJ 3rd. Molecular markers in thyroid cancer diagnostics. Surg Clin North Am. 2009; 89 (5):1139-55. doi: 10.1016/j.suc.2009.06.012. PMID: 19836489.

14 Eszlinger M, Paschke R. Molecular fine-needle aspiration biopsy diagnosis of thyroid nodules by tumor specific mutations and gene expression patterns. Mol Cell Endocrinol. 2010; 322 (1-2):29-37. doi: 10.1016/j.mce.2010.01.010. PMID: 20083161.

15 Yuan L, Nasr C, Bena JF, Elsheikh TM. Hürthle cell-predominant thyroid fine needle aspiration cytology: A four risk-factor model highly accurate in excluding malignancy and predicting neoplasm. Diagn Cytopathol. 2022; 50 (9):424-435. doi: 10.1002/dc.25000. PMID: 35674254; PMCID: PMC9543473.

16 McKee S, Wu H, Wang X, Cramer H, Lin J, Chen S; Hürthle Cell Neoplasms Diagnosed by Fine Needle Aspiration Are Not Associated with an Increased Risk of Malignancy. Acta Cytologica. 2014; 58 (3): 235–238. doi: 10.1159/000361073.

17 de Micco C, Savchenko V, Giorgi R, Sebag F, Henry JF. Utility of malignancy markers in fine-needle aspiration cytology of thyroid nodules: comparison of Hector Battifora mesothelial antigen-1, thyroid peroxidase and dipeptidyl aminopeptidase IV. Br J Cancer. 2008; 98 (4):818-23. doi: 10.1038/sj.bjc.6604194. PMID: 18212751; PMCID: PMC2259194.

18 Mijovic T, Gologan O, Rochon L, Hier M, Black MJ, Young J, Rivera J, Tamilia M, Payne RJ. Fine-needle aspiration biopsy of the thyroid: review of cytopathologic features predictive of malignancy. J Otolaryngol Head Neck Surg. 2009; 38 (3):348-54. PMID: 19476767.

19 Ivanova R, Soares P, Castro P, Sobrinho-Simões M. Diffuse (or multinodular) follicular variant of papillary thyroid carcinoma: a clinicopathologic and immunohistochemical analysis of ten cases of an aggressive form of differentiated thyroid carcinoma. Virchows Arch. 2002; 440 (4):418-24. doi: 10.1007/s00428-001-0543-3. PMID: 11956824.

20 Rossi ED, Raffaelli M, Minimo C, Mule A, Lombardi CP, Vecchio FM, Fadda G. Immunocytochemical evaluation of thyroid neoplasms on thin-layer smears from fine-needle aspiration biopsies. Cancer. 2005; 105 (2):87-95. doi: 10.1002/cncr.21026. PMID: 15742329.

21 Bartolazzi A, Gasbarri A, Papotti M, Bussolati G, Lucante T, Khan A, Inohara H, Marandino F, Orlandi F, Nardi F, Vecchione A, Tecce R, Larsson O; Thyroid Cancer Study Group. Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions. Lancet. 2001; 357 (9269):1644-50. doi: 10.1016/s0140-6736 (00)04817-0. PMID: 11425367.

22 Nergård-Nilssen T, Hulme C. Developmental dyslexia in adults: behavioural manifestations and cognitive correlates. Dyslexia. 2014; 20 (3):191-207. doi: 10.1002/dys.1477. PMID: 24842581.

23 Collet JF, Hurbain I, Prengel C, Utzmann O, Scetbon F, Bernaudin JF, Fajac A. Galectin-3 immunodetection in follicular thyroid neoplasms: a prospective study on fine-needle aspiration samples. Br J Cancer. 2005; 93 (10):1175-81. doi: 10.1038/sj.bjc.6602822. PMID: 16251880; PMCID: PMC2361502.

24 Kim MJ, Kim HJ, Hong SJ, Shong YK, Gong G. Diagnostic utility of galectin-3 in aspirates of thyroid follicular lesions. Acta Cytol. 2006; 50 (1):28-34. doi: 10.1159/000325891. PMID: 16514837.

25 Carpi A, Naccarato AG, Iervasi G, Nicolini A, Bevilacqua G, Viacava P, Collecchi P, Lavra L, Marchetti C, Sciacchitano S, Bartolazzi A. Large needle aspiration biopsy and galectin-3 determination in selected thyroid nodules with indeterminate FNA-cytology. Br J Cancer. 2006; 95 (2):204-9. doi: 10.1038/sj.bjc.6603232. PMID: 16804521; PMCID: PMC2360621.

26 Giovanella L, Crippa S, Cariani L. Serum calcitonin-negative medullary thyroid carcinoma: role of CgA and CEA as complementary markers. Int J Biol Markers. 2008; 23 (2):129-31. doi: 10.1177/172460080802300212. PMID: 18629788.

27 Jain R, Fischer S, Serra S, Chetty R. The use of Cytokeratin 19 (CK19) immunohistochemistry in lesions of the pancreas, gastrointestinal tract, and liver. Appl Immunohistochem Mol Morphol. 2010; 18 (1):9-15. doi: 10.1097/PAI.0b013e3181ad36ea. PMID: 19956064.

28 Ito Y, Yoshida H, Tomoda C, Uruno T, Miya A, Kobayashi K, Matsuzuka F, Kakudo K, Kuma K, Miyauchi A. S100A4 expression is an early event of papillary carcinoma of the thyroid. Oncology. 2004;67 (5-6):397-402. doi: 10.1159/000082924. PMID: 15713996.

29 Ding Z, Ke R, Zhang Y, Fan Y, Fan J. FOXE1 inhibits cell proliferation, migration and invasion of papillary thyroid cancer by regulating PDGFA. Mol Cell Endocrinol. 2019; 493:110420. Mol Cell Endocrinol. 2022; 549:111640. doi: 10.1016/j.mce.2019.03.010. PMID: 31129275.

30 Mond M, Bullock M, Yao Y, Clifton-Bligh RJ, Gilfillan C, Fuller PJ. Somatic Mutations of FOXE1 in Papillary Thyroid Cancer. Thyroid. 2015; 25 (8):904-10. doi: 10.1089/thy.2015.0030. PMID: 25950909.

31 Bartolazzi A, Orlandi F, Saggiorato E, Volante M, Arecco F, Rossetto R and et al; Italian Thyroid Cancer Study Group (ITCSG). Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: a prospective multicentre study. Lancet Oncol. 2008; 9 (6):543-9. doi: 10.1016/S1470-2045 (08)70132-3. PMID: 18495537.

32 Pennelli G, Mian C, Pelizzo MR, Naccamulli D, Piotto A, Girelli ME, and et al. Galectin-3 cytotest

in thyroid follicular neoplasia: a prospective, monoinstitutional study. Acta Cytol. 2009; 53 (5):533-9. doi: 10.1159/000325381. PMID: 19798881.

33 Bonzanini M, Amadori PL, Sagramoso C, Dalla Palma P. Expression of cytokeratin 19 and protein p63 in fine needle aspiration biopsy of papillary thyroid carcinoma. Acta Cytol. 2008; 52 (5):541-8. doi: 10.1159/000325595. PMID: 18833815.

34 Mase T, Funahashi H, Koshikawa T, Imai T, Nara Y, Tanaka Y, Nakao A. HBME-1 immunostaining in thyroid tumors especially in follicular neoplasm. Endocr J. 2003; 50 (2):173-7. doi: 10.1507/endocrj.50.173. PMID: 12803237.

35 Ozolins A, Narbuts Z, Strumfa I, Volanska G, Gardovskis J. Diagnostic utility of immunohistochemical panel in various thyroid pathologies. Langenbecks Arch Surg. 2010; 395 (7):885-91. doi: 10.1007/s00423-010-0690-6. PMID: 20640858.

36 Busnardo B, Girelli ME, Simioni N, Nacamulli D, Busetto E. Nonparallel patterns of calcitonin and carcinoembryonic antigen levels in the follow-up of medullary thyroid carcinoma. Cancer. 1984; 53 (2):278-85. doi: 10.1002/1097-0142 (19840115)53:2<278:aid-cncr2820530216>3.0.co;2-z. PMID: 6690009.

37 Baquero P, Sánchez-Hernández I, Jiménez-Mora E, Orgaz JL, Jiménez B, Chiloeches A. (V600E) BRAF promotes invasiveness of thyroid cancer cells by decreasing E-cadherin expression through a Snail-dependent mechanism. Cancer Lett. 2013; 335 (1):232-41. doi: 10.1016/j.canlet.2013.02.033. PMID: 23435375.

38 McFadden DG, Vernon A, Santiago PM, Martinez-McFaline R, Bhutkar A, Crowley DM, McMahon M, Sadow PM, Jacks T. p53 constrains progression to anaplastic thyroid carcinoma in a Braf-mutant mouse model of papillary thyroid cancer. Proc Natl Acad Sci U S A. 2014; 111 (16): E1600-9. doi: 10.1073/pnas.1404357111. PMID: 24711431; PMCID: PMC4000830.

39 Torregrossa L, Faviana P, Filice ME, Materazzi G, Miccoli P, Vitti P, Fontanini G, Melillo RM, Santoro M, Basolo F. CXC chemokine receptor 4 immunodetection in the follicular variant of papillary thyroid carcinoma: comparison to galectin-3 and hector battifora mesothelial cell-1. Thyroid. 2010; 20 (5):495-504. doi: 10.1089/thy.2009.0282. PMID: 20450430.

40 Schmitt AC, Cohen C, Siddiqui MT. Paired box gene 8, HBME-1, and cytokeratin 19 expression in preoperative fine-needle aspiration of papillary thyroid carcinoma: diagnostic utility. Cancer Cytopathol. 2010; 118 (4):196-202. doi: 10.1002/cncy.20082. PMID: 20731005.