Expression of Galectin 3, HBME1 and CK19 in Benign and Malignant Thyroid Lesions

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Abstract

Background: The accurate diagnosis of well differentiated thyroid tumors is very important for clinical management of patients. Circumscribed lesions having follicular pattern of growth like encapsulated papillary thyroid carcinoma (follicular variant) and microinvasive follicular thyroid carcinoma can cause lots of diagnostic problems in their distinction from benign lesions as follicular adenoma and hyperplastic thyroid nodules with abnormal nuclear features.

Aim: To assess the utility of the immunohistochemical markers (CK 19, HBME 1, and Galectin 3) either used singly or in combination to differentiate benign and malignant thyroid lesions, especially well differentiated papillary and follicular carcinoma from follicular adenoma.

Material and Methods: A total of forty specimens were retrospectively reviewed in this study, including 33 thyroidectomies, 4 neck lymph node excisional biopsies showing metastatic deposits and 3 core tissue biopsies from bone metastatic deposits. Four (5 microns thick) sections were prepared from each tissue block, one stained with hematoxylin and eosin and the others were subjected to the three immunohistochemical markers (CK 19, HBME 1, and Galectin 3).

Results: CK 19 stained positively 95% of papillary carcinoma cases 40% of follicular carcinoma cases and 50% of follicular adenoma cases. HBME 1 stained 90% of papillary carcinoma cases, 50% of follicular carcinoma cases, and only 10% of follicular adenoma cases. Galectin 3 stained positively 100% of papillary carcinoma cases, 20% of follicular carcinoma cases and 20% of follicular adenoma cases. These results proved that HBME1 is the most valuable marker if used alone in the differential diagnosis of papillary carcinoma versus follicular adenoma.

Conclusion: These results proved that a panel of CK 19, HBME 1 and Galectin 3 is very useful in differentiating papillary thyroid carcinoma from follicular adenoma especially in problematic cases when the nuclear features can not be conclusive alone.

Key Words: Malignant thyroid lesions – Benign thyroid lesions – CK19 – HBME1 – Galectin 3

Introduction

THYROID nodules are extremely common and usually discovered during routine medical care, with an estimation of 7% of the general population develops clinically palpable thyroid nodules [1].

The macroscopic appearance of the normal adult thyroid gland is that of a bilobate organ in the mid portion of the neck, immediately in front of the larynx and trachea. The two lobes are joined by the isthmus. The normal weight of the adult gland ranges from 15 to 25 grams. The gland is generally larger and heavier in women than in men and changes during pregnancy and the menstrual cycle, increasing up to 50 percent during the early secretory phase of the cycle [2].

The thyroid gland is divided into lobules composed of 20 to 40 follicles supplied by a branch of the thyroid artery. Each follicle can range from 50 to 500µm, with an average size of 200µm. Follicles contain colloid in their Lumina which consists of concentrated Periodic Acid Schiff (PAS)-positive thyroglobulin. The tinctorial quality of the colloid may vary with the activity of the follicles, i.e., active follicles usually have weakly eosinophilic and flocculent colloid while inactive follicles tend to have more eosinophilic colloid [3].

The accurate diagnosis of well differentiated thyroid tumors is very important for clinical management of patients [4].

In the majority of cases, the pathological diagnosis of surgically removed thyroid nodules is done by routine Hematoxylin and Eosin (HE) staining. However, there are cases which does not allow the differentiation between benign and malignant lesions by the pathological criteria [5].
Circumscribed lesions having follicular pattern of growth like encapsulated papillary thyroid carcinoma (follicular variant) and micro invasive follicular thyroid carcinoma can cause lots diagnostic problems in their distinction from benign lesions as follicular adenoma and hyperplastic thyroid nodules with abnormal nuclear features [6].

On the other hand, the entities described by Williams (2000) [7] called "Follicular tumor of undetermined malignant potential" and "Well differentiated tumor of undetermined malignant potential" cause lots of confusion for clinicians.

Even Grave’s disease that may coexist with all types of thyroid cancer, especially papillary carcinoma, can show foci having vesicular nuclei and papillary formations, which makes the differential diagnosis between a true papillary carcinoma and foci mimicking papillary carcinoma very challenging by light microscopic features only [8].

WHO histological classification of thyroid tumors (2004) [9].

**Thyroid carcinomas:**
- Papillary carcinoma.
- Follicular carcinoma.
- Poorly differentiated carcinoma (insular carcinoma).
- Undifferentiated (anaplastic) carcinoma.
- Squamous cell carcinoma.
- Mucoepidermoid carcinoma.
- Sclerosing mucoepidermoid carcinoma with eosinophilia.
- Mucinous carcinoma.
- Medullary carcinoma.
- Mixed medullary and follicular carcinoma.
- Carcinoma showing thymus like differentiation.

**Thyroid adenomas and related tumors:**
- Follicular adenoma.
- Hyalinizing trabecular tumor.

**Other thyroid tumors:**
- Teratoma.
- Primary lymphoma and plasmacytoma.
- Ectopic thymoma.
- Angiosarcoma.
- Smooth muscle tumors.

- Peripheral nerve sheath tumors.
- Parangangioma.
- Solitary fibrous tumor.
- Follicular dendritic cell tumor.
- Langerhans cell histiocytosis.

**Secondary tumors:**
Galectin-3 is a member of the lectin family. It is approximately 30kDa. Galectin-3 is encoded by a single gene, LGALS3, located on chromosome 14, locus q21-q22 [10]. It is expressed in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space [11]. This protein has been shown to be involved in the following biological processes: Cell adhesion, cell activation and chemo-attraction, cell growth and differentiation, cell cycle, and apoptosis. Given galectin-3’s broad biological functionality, it has been demonstrated to be involved in cancer, inflammation and fibrosis, heart disease, and stroke [12].

Several investigators have found Galectin 3 expression to be of value in discriminating between benign and malignant thyroid nodules [13] as LGALS3 gene was found to be upregulated in papillary thyroid carcinoma compared to normal thyroid [14].

HBME-1 is a monoclonal antibody generated against a suspension of malignant epithelial mesothelioma cells; it reacts with the micro villous surface protein of mesothelial cells. HBME1 expression has been reported in papillary and follicular thyroid carcinoma but not in normal thyroid cells [15].

Cytokeratin 19 (CK19) is a member of the keratin family. The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells and are subdivided into cytkeratins and hair keratins [16].

KRT19 gene was found to be upregulated in papillary thyroid carcinoma compared to normal thyroid [14], so this cyto-skeletal protein (CK19) is significantly increased in papillary thyroid carcinoma; but this finding remains controversial since it is also positive in chronic lymphocytic thyroiditis and at sites of reaction, usually in response to previous aspiration biopsy [17].

**Material and Methods**

**Case selection:** A total of forty specimens were retrospectively reviewed in this study, including 33 thyroidectomies, 4 neck lymph node excisional
biopsies showing metastatic deposits and 3 core tissue biopsies from bone metastatic deposits. The specimens were obtained from the Department of Pathology, Faculty of Medicine, Cairo University and from other private laboratories during the period from January 2009 to September 2009.

1- Thyroidectomy specimens: 33 cases were examined and staged according to the TNM staging system.

2- Lymph node excisional biopsies: 4 cases were examined and proved to be of Papillary thyroid origin based on the typical papillary architecture and the typical nuclear features of papillary thyroid carcinoma.

3- Specimens from bony metastatic deposits: 3 cases were examined and proved to be of thyroid origin by immunostaining against thyroglobulin antibody (DAKO).

Histological review: Four (5 microns thick) sections were prepared from each tissue block, one of them stained by Hematoxylin and Eosin (H & E) for histological re-evaluation; and the other three were mounted on poly-L-lysine-coated slides (Superfrost slides) and subjected to the three immunohistochemical markers (CK19, HBME-1, and Galectin 3).

Evaluation of CK 19 expression: Cytoplasmic staining in more than 10% of cells was considered positive.

Evaluation of HBME-1 expression: Cytoplasmic and cell membrane staining in more than 10% of cells was considered positive.

Evaluation of galectin 3 expression: Cytoplasmic and nuclear staining in more than 10% of cells was considered positive.

Statistical analysis:
Statistical analyses were done using the Fisher exact test. A $p$-value of <0.05 was considered statistically significant.

Results

Forty cases of papillary thyroid carcinomas, follicular thyroid carcinomas and follicular adenomas were studied in this thesis.

Morphology:

Papillary thyroid carcinoma cases: A total of twenty cases had a final diagnosis of papillary thyroid carcinoma following evaluation of the Haematoxylin and Eosin stained sections including:

<table>
<thead>
<tr>
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<th>Papillary carcinoma</th>
<th>Follicular carcinoma</th>
<th>Follicular adenoma</th>
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</thead>
<tbody>
<tr>
<td>CK 19 +ve</td>
<td>19 (95%)</td>
<td>4 (40%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>CK 19 –ve</td>
<td>1 (5%)</td>
<td>6 (60%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
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</table>

The 16 thyroidectomy cases were staged according to the TNM staging system and revealed: One case was staged as T1, nine cases as T2 and 6 cases as T3. Only six of the thyroidectomy specimens were accompanied by neck node sampling, five of them revealed positive metastatic deposits.

Different histological types were encountered: 14 were conventional papillary carcinomas (three of them were encapsulated), four were follicular variant, and two were tall cell variant.

The age of the patients ranged from 18 to 59 with a median age of 34. Male to female ratio was 1: 1.8.

Follicular carcinoma cases: A total of ten cases had a final diagnosis of follicular thyroid carcinoma following evaluation of the Haematoxylin and Eosin stained sections including: 7 cases encountered in thyroidectomy specimens and three cases in core biopsies from metastasis in bone.

The 7 thyroidectomy cases were staged according to the TNM staging system and revealed: 3 cases as T2 and 4 cases as T3.

The age of the patients ranged from 39 to 64 with a median age of 50. Male to female ratio was 1: 1.5.

Follicular adenoma cases: A total of ten cases had a final diagnosis of follicular adenoma following evaluation of the Haematoxylin and Eosin stained sections, all were encountered in thyroidectomy specimens.

Different histological patterns were detected as follows: Four showed normofollicular, two microfollicular and four macrofollicular patterns. No unusual variants encountered.

The age of the patients ranged from 22 to 52 with a median age of 38. Male to female ratio was 1: 2.3.

Cytokeratin 19 (CK19) expression: Table (1) shows CK 19 expression, in papillary carcinoma, follicular carcinoma and follicular adenoma.
The relation of CK 19 expression between papillary carcinoma and follicular adenoma, and papillary carcinoma and follicular carcinoma was statistically significant \( p=0.01768 \) and 0.004244 respectively. While the relation between follicular carcinoma and follicular adenoma was insignificant \( p>0.9999 \).

**HBME-1 expression:** Table (2) shows HBME-1 expression, in papillary carcinoma, follicular carcinoma and follicular adenoma.

<table>
<thead>
<tr>
<th></th>
<th>Papillary carcinoma</th>
<th>Follicular carcinoma</th>
<th>Follicular adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBME-1 +ve</td>
<td>18 (90%)</td>
<td>5 (50%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>HBME-1 –ve</td>
<td>2 (10%)</td>
<td>5 (50%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
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</table>

The relation of HBME-1 expression was statistically highly significant between only papillary carcinoma and follicular adenoma \( p=0.0000702 \), while it was statistically insignificant between papillary carcinoma and follicular carcinoma \( p=0.0512 \), and between follicular carcinoma and follicular adenoma was insignificant \( p=0.14 \).

**Galectin 3 expression:** Table (3) shows Galectin 3 expression, in papillary carcinoma, follicular carcinoma and follicular adenoma.

<table>
<thead>
<tr>
<th></th>
<th>Papillary carcinoma</th>
<th>Follicular carcinoma</th>
<th>Follicular adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectin 3 +ve</td>
<td>20 (100%)</td>
<td>2 (20%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Galectin 3 –ve</td>
<td>0</td>
<td>8 (80%)</td>
<td>8 (80%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
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</table>

The relation of Galectin 3 expression between papillary carcinoma and follicular adenoma, and papillary carcinoma and follicular carcinoma was statistically highly significant \( p=0.000015 \) and 0.0000153 respectively. While the relation between follicular carcinoma and follicular adenoma was insignificant \( p=1.41 \).

It is important to note that all histiocytes detected in any of the three tumors described above were positively stained by Galectin 3; so great caution has to be taken during assessment of the percentage of positively stained cells to avoid inclusion of the histiocytic cells with the epithelial cells.

**Combinations of all three markers:**

Table (4): Co-expression of HBME-1, Galectin 3, and CK19 in both papillary carcinoma and follicular adenoma.

<table>
<thead>
<tr>
<th></th>
<th>Papillary carcinoma</th>
<th>Follicular adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBME-1 (+) and Galectin 3 (+) and CK19 (+)</td>
<td>17 (85%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>- Other</td>
<td>3 (15%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>- Total</td>
<td>20 (100%)</td>
<td>10 (100%)</td>
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</tbody>
</table>

CK19 : Cytokeratin 19.
Other : Either HBME-1 or Galectin 3 or CK19 positive, or only two of them positive, or all markers negative.
\[ p = 0.0001904, \text{statistically highly significant.} \]

Table (5): Co-expression of HBME-1, Galectin 3, and CK19 in both papillary carcinoma and follicular carcinoma.

<table>
<thead>
<tr>
<th></th>
<th>Papillary carcinoma</th>
<th>Follicular adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBME-1 (+) and Galectin 3 (+) and CK19 (+)</td>
<td>17 (85%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>- Other</td>
<td>3 (15%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>- Total</td>
<td>20 (100%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

CK19 : Cytokeratin 19.
Other : Either HBME-1 or Galectin 3 or CK19 positive, or only two of them positive, or all markers negative.
\[ p = 0.0002680, \text{statistically significant.} \]

Table (6): Co-expression of HBME-1 and Galectin 3 in both follicular carcinoma and follicular adenoma.

<table>
<thead>
<tr>
<th></th>
<th>Follicular carcinoma</th>
<th>Follicular adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBME-1 (+) and Galectin 3 (+)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (90%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20 (100%)</td>
<td>10 (100%)</td>
</tr>
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</table>

Other: Either HBME-1 or Galectin 3 positive, or both markers negative \( p>0.9999999, \text{statistically not significant.} \)

Table (7): Sensitivity and specificity of papillary thyroid carcinoma vs Follicular adenoma for each of the used immunohistochemical markers and their combinations.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>HBME-1</td>
<td>90%</td>
<td>90%</td>
</tr>
<tr>
<td>Galectin 3</td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>CK 19</td>
<td>95%</td>
<td>50%</td>
</tr>
<tr>
<td>Coexpression of HBME-1 and Galectin 3,</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>Coexpression of HBME-1, Galectin 3 and CK19.</td>
<td>85%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Coexpression: Both or all markers used are positive at the same time.
Table (8): Sensitivity and specificity of thyroid carcinomas (papillary and follicular) vs follicular adenoma for each of the used immunohistochemical markers and their combinations.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>- HBME-1</td>
<td>77%</td>
<td>90%</td>
</tr>
<tr>
<td>- Galectin 3</td>
<td>73%</td>
<td>80%</td>
</tr>
<tr>
<td>- CK 19</td>
<td>77%</td>
<td>50%</td>
</tr>
<tr>
<td>- Coexpression of HBME-1 and Galectin 3</td>
<td>63%</td>
<td>100%</td>
</tr>
<tr>
<td>- Coexpression of HBME-1, Galectin 3 and CK19</td>
<td>60%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Coexpression: Both or all markers used are positive at the same time.

Fig. (1): Typical nuclear features of papillary thyroid carcinoma with nuclear grooves (yellow arrow) and nuclear pseudo-inclusions (arrow heads) (H & E X 1000).

Fig. (2): Case of papillary carcinoma conventional and follicular type (A), strongly positive for CK 19 (B), positive for HBME-1 (C) and strongly positive for Galectin 3 (D) (X 40).
Fig. (3): Vascular invasion in a follicular carcinoma (A) showing focal weak positivity for CK19 (B), strong positivity for HBME-1 (C) and total negativity for Galectin 3 (D) (X 200).

N.B.: Fig. (4-C): Shows the typical baso-lateral pattern of staining against HBME-1.

Fig. (4): Follicular adenoma with normo-follicular pattern of growth (A), focally positive for CK19 (B), negative for HBME-1 (C) and negative for Galectin 3 (D) (X 400).
Discussion

One of the most frequent difficulties in thyroid pathology is differentiating adenomas from carcinomas, especially those with follicular architecture.

Examples of problematic cases: Cases of follicular adenoma that show large and pale (Blown up) nuclei, mimicking the nuclear features of papillary carcinoma; other cases described as "Follicular adenoma with papillary hyperplasia or papillary variant of follicular adenoma" show diagnostic difficulties especially when some of the nuclei show enlargement or clearing. In addition, the entity described by Williams, named "Well differentiated tumor of uncertain malignant potential", having limited evidence of unequivocal capsular or vascular invasion, together with small foci showing nuclei similar to those encountered in papillary carcinoma, make great diagnostic problems.

This differentiation (Adenoma versus Carcinoma) is critical for the treatment and long-term management of the tumors; also the differentiation (Papillary versus follicular carcinoma) has great prognostic importance.

As previously mentioned, antibodies against CK19 and Galectin 3 were selected for this study because their genes were found to be consistently up regulated in papillary thyroid carcinoma compared to normal thyroid. The monoclonal antibody HBME-1 was included for comparison because of its reported specificity for papillary thyroid carcinoma.

In this study, CK 19 was expressed in 19 out of 20 cases of papillary carcinoma with 95% sensitivity. This very high sensitivity agrees with the results stated by Scognamiglio et al., [20] as CK19 stained 75 out of 78 papillary carcinoma cases with 96% sensitivity. Slightly lower sensitivity of 90% and 85% was found respectively by Park et al., [21] and Saleh et al., [22].

On the contrary, CK 19 was the least specific marker for papillary carcinoma in our study by staining 5 out of 10 follicular adenomas (specificity 50%); although it usually stained lesser percentage of cells in the follicular adenoma than the papillary carcinoma and with a lesser intensity. Results of the studies derived by Nasr et al., [6] and Saleh et al., [22] showed similar CK 19 specificity (32% and 50% respectively), but Prasad et al., [23], and Park et al., [21] studies, showed a specificity of 90%, and 83% respectively. The reason of this great difference might be because of the very small number of adenoma cases [5] used in Prasad's [23] study and the usage of different CK 19 antibody clone (RCK108; 1: 150; DakoCytomation) by Park et al., [21]. This low specificity makes CK 19 an unreliable marker, if used alone, in the differential diagnosis of papillary carcinoma versus follicular adenoma.

Four out of our ten follicular carcinoma cases were positive for CK19 in comparison with 5 positive follicular adenomas out of 10; this was statistically not significant (p>0.999) making the distinction between these two entities by this marker not possible. Similar conclusions are also reached by Saleh et al., [22].

The specificity of CK 19 expression in differentiating papillary and follicular carcinomas was also low (60%), this meets the results of Prasad et al., [23] and Park et al., [21] about this issue; again, CK19 is not a suitable marker to be used alone in such cases.

Galectin 3 is a controversial marker well studied in the last ten years in different thyroid lesions. In our study it was expressed in all papillary carcinoma cases (20/20) with a sensitivity of 100%. Studies done by Prasad et al., [23], Scognamiglio et al., [20] and Park et al., [21] showed similar results with a sensitivity exceeding 92%.

Specificity of Galectin 3 in the differential diagnosis of thyroid carcinomas (papillary and follicular) versus follicular adenoma was relatively low (80%) in our study as it was expressed in 2 out of 10 follicular adenomas. Scognamiglio et al., [20] showed also a Galectin 3 specificity of 82%. Much lower specificity (58%) was found by Saleh et al., [22] as Galectin 3 stained 19 out of their 46 follicular adenoma cases, even if their staining pattern was focal and weak. This great difference might be explained by mentioning that Saleh et al., [22] included cases of hyalinizing trabecular adenoma with the "follicular adenoma group" in their study. Based on the great-mentioned-genetic and phenotypic similarities between papillary carcinoma and hyalinizing trabecular adenoma, we can suspect that many of these hyalinizing trabecular adenomas were positive for Galectin 3, thus reducing significantly it's specificity in their study. Saleh et al., [22] themselves tried to explain their results by stating a fact telling that follicular cells normally contain endogenous "biotin" (used in the detection kits of the Immunostains in general) that can cause false Galectin 3 positivity. Therefore they suggested that the usage of Galectin 3 is preferred with a "biotin" free
detection system. Kovacs et al., [24] also indicated that there might be some interpretation problems caused by observation of focal positivity in inflammatory and cystic lesions; they postulated that expression of Galectin 3 in non neoplastic follicular cells in inflamed areas may result from cytokines scattered by the inflammatory cells or simple permeation of Galectin 3 abundantly shed by lymphocytes in the neighboring follicular cells.

Strong positive Galectin 3 staining of macrophages and other inflammatory cells was detected in the different lesions we studied confirming the findings of Kovacs et al., [24]. This finding makes lots of limitations on any trial to use this marker to differentiate malignant from benign lesions in fine needle aspiration samples.

On the other hand, Adela et al., [1], stated that none of the benign thyroid cases stained positive for CK and Gal-3, making these two antibodies 100% specific for differentiating benign thyroid lesions from papillary thyroid carcinoma.

In the purpose of differentiating papillary from follicular carcinoma, Galectin 3 showed a specificity of 80% in the present study as it stained only two out of the 10 follicular carcinoma cases. Prasad et al., [23] and Park et al., [21] showed a much lower specificity, 33% and 64% respectively. This big difference in the results can be attributed to the small number of follicular carcinomas in our study. So, the use of Galectin 3 as a single marker to differentiate papillary from follicular carcinoma is not recommended.

In the current study, HBME-1 was the most sensitive marker for thyroid carcinomas (papillary and follicular carcinomas together), it was positive in 23 out of 30 carcinoma cases (sensitivity 77%). The study done by Adela et al., [1] showed a lower level 63.6%. The study done by Saleh et al., [22] showed a slightly higher sensitivity (87%) because this marker was expressed in 18 out of 22 follicular carcinoma cases (in addition to its expression in most of their papillary carcinoma cases); but in our study it stained only 5 out of 10 follicular carcinomas. This slight difference can be attributed also to the relatively small number of follicular carcinomas in our study.

The specificity of HBME-1 concerning the differentiation between well differentiated malignant and benign thyroid neoplasms varies greatly from study to another. In our study the specificity was 90%, in Nasr’s [6] it was 100%, in Scognamiglio's et al., [20] it was 96%, and in Prasad's et al., [23] it was 90%. Park et al., [21] and Saleh et al., [22] showed totally different results; the specificity of HBME-1 in their studies was 68.5% and 43% respectively. This can be explained by the criteria Nasr et al., [6] recommended for the interpretation of its staining: "HBME-1 must stain the "basolateral" membrane of the cell in order to be considered positive"; when this criterion is strictly applied, positive HBME-1 staining is highly specific for papillary thyroid carcinoma. So, most probably studies that showed low specificity of HBME-1 for papillary carcinoma did not respect the rules suggested by Nasr et al., [6] in interpreting the staining results.

According to these results we can conclude that HBME-1 is a reliable marker (with a relatively high sensitivity and a high specificity) in differentiating papillary carcinoma from follicular adenoma and also in differentiating well differentiated thyroid carcinomas (Papillary and follicular) in general from follicular adenoma, especially when using the strict Nasr's criteria in the interpretation of its staining pattern.

Co-expression of the used markers was studied to see if the use of these markers together will affect the sensitivity or the specificity in the differential diagnosis of papillary thyroid carcinoma versus follicular adenoma or that of thyroid carcinomas (papillary and follicular) versus follicular adenoma. It was found that the co-expression of HBME-1 and Galectin 3 was highly significant statistically; it had high sensitivity (90%) and increased significantly the specificity (100%). Scognamiglio et al., [20] showed similar results as the co-expression of these two markers was 86% sensitive and 96% specific for papillary carcinoma. Also in Park et al., [21] results, the sensitivity was 84% and the specificity was 100% which is a higher specificity than that of any immuno-marker used alone.

The addition of CK 19 to these two markers and assessment of the co-expression of all three used markers together decreases slightly the sensitivity (85%), but the specificity was still (100%). Scognamiglio et al., [20] proved that adding CK19 positivity to the positivity of both HBME-1 and Galectin 3 increases the specificity from 96% to 100% in differentiating papillary carcinoma from follicular adenoma. Similarly Park et al., [21] reported that this combination has a specificity of 100%. Surprisingly, Saleh et al., [22] stated that although this combination has a relatively high sensitivity (86%), it has low specificity (68%). This discrepancy is mostly related to the previously
mentioned problem in interpretation of HBME-1 staining, especially in follicular adenomas. So, it is highly recommended to use at least two markers and better all three markers in combination to attain better accuracy in differentiating papillary carcinoma from follicular adenoma.

In our study, not a single papillary thyroid carcinoma was negative for all markers together or even positive for only one marker. On the other hand 3 out of the 10 follicular adenomas were totally negative for all three markers, 6 were positive for only one marker and only one was positive for 2 markers (CK19 and HBME-1). Prasad et al., [26] found that total negativity of all markers was highly sensitive and highly specific for adenoma. This finding can have an important clinical significance: If multiple core biopsies were taken from a clinically suspicious solitary thyroid nodule and subjected to these three immunohistochemical markers (CK19, HBME-1 and Galectin 3) and their results were totally negative, this means that this lesion is mostly a benign adenoma; so, we can avoid many unneeded surgical interventions.

Interestingly the co-expression of both HBME-1 and Galectin 3 only or the co-expression of all our three markers together was statistically highly significant concerning the differentiation between papillary and follicular carcinoma with a sensitivity of 85% and a specificity of 90%, which may help in discriminating lesions having follicular pattern of growth with questionable nuclear features.

The comparison of expression of the three used markers and their combinations in both follicular adenoma and follicular carcinoma was statistically not significant. Similar results were stated by Park et al., [21] and Saleh et al., [22]. This means that it is preferable to stick to the histological criteria (presence or absence of capsular and/or vascular invasion) to accurately differentiate these two entities.

Conclusions and recommendations:

An immunohistochemical panel including CK19, HBME1, and Galectin 3 is very useful in the differential diagnosis: Papillary carcinoma, follicular carcinoma and follicular adenoma; thus it will be useful in reaching a definite diagnosis in problematic thyroid nodules with follicular pattern of growth and equivocal capsular or vascular invasion or equivocal nuclear features.

Further studies are recommended to try to identify a totally specific, highly sensitive marker for papillary and follicular carcinomas.

References

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