Detection of HBME-1 and Galectin-3 by Immunohistochemistry in Follicular Lesions

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Abstract

The main problem established by a discovery of a thyroid nodule is to discriminate between a benign and malignant lesion. Differential diagnosis between follicular thyroid cancer (FTC) and benign follicular thyroid adenoma (FTA) is a great challenge for even an experienced pathologist and requires special effort. A developing number of some encouraging IHC markers for the differential diagnosis of thyroid lesions have emerged, including, Hector Battifora mesothelial (HBME-1) and galectin-3 (Gal-3). There was significant positive correlation between Galectin-3 and HBME-1 in follicular carcinoma and follicular variant of papillary carcinoma (r= 0.380, P= 0.041) and (r= 0.315, P=0.047) respectively. There was no significant correlation between Galectin-3 and HBME-1 in follicular adenoma and follicular hyperplasia. Immunohistochemical expression of Galectin-3(Gal-3) there was highly significant difference (P<0.001) among study groups (FC, FVPC, FA, follicular hyperplasia) while there was no significant difference in mean of immunohistochemical score of Galectin-3 between follicular carcinoma, follicular variant of and papillary carcinoma (P>0.05); however, carcinoma of both types showed significantly higher Galectin-3 score than both follicular adenoma and follicular hyperplasia (P<0.001). In addition, the score of follicular adenoma was significantly greater than that of follicular hyperplasia (P<0.05). Immunohistochemical expression of HBME-1 immunohistochemical expression of HBME-1 was highly significance among study groups (FC, FVPC, FA, follicular hyperplasia) while there was no significant difference in mean score between follicular carcinoma and follicular variant of papillary carcinoma (P>0.05); however, carcinoma of both types showed significantly higher HBME-1 score than both follicular adenoma and follicular hyperplasia (P<0.001). In addition, the score of follicular adenoma was significantly greater than that of follicular hyperplasia (P<0.05).

Keywords: Galectin-3, HBME-1, Thyroid.

Introduction

Thyroid cancer is a common endocrine malignancy. There has been exciting progress in understanding its molecular pathogenesis in recent years, as best exemplified by the elucidation of the fundamental role of several major signaling pathways and related molecular derangements [1].

Nodular follicular lesions of thyroid gland comprise benign and malignant neoplasm. Nodular follicular thyroid lesions have in common many morphological features, therefore attempts were made to define additional criteria for distinction between follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma. Increasing number of Immunohistochemistry (IHC) markers Hector Battifora Mesothelium (HBME-1) Galectin-3(Gal-3), (CK19), CD56 and p63 in the continual process of evaluation [2].

The differential diagnosis of an obvious thyroid nodule includes a dominant or first nodule of a multinodular goiter, benign adenomas, thyroid cysts, focal thyroiditis and carcinoma. The nodules of a multinodular goiter are polyclonal are not considered to
have an increased risk of malignancy. The etiology of benign adenomas is unknown but is clearly monoclonal in origin. Thyroid adenoma may be hyper functioning causing thyrotoxosis [3]. The main problem established by a discovery of a thyroid nodule is to discriminate between a benign and malignant lesion [4].

As the monoclonal antibody Hector Battifora Mesothelial (HBME-1) produced by mesothelial cells, it reacts with an unknown antigen present in the microvillus surface of normal and neoplastic mesothelial cells, has been appeared to have reactivity in thyroid carcinomas, the diagnostic usefulness of marker HBME-1 in follicular neoplasms [5]. Galectin-3(Gal-3) a member of the beta-galactosidase binding family of lectins has been viewed as a helpful tool for discriminating malignant tumors from benign nodules of the thyroid, including the distinction between follicular carcinoma and adenoma [6].

However, there are follicular tumors with unclear vascular or capsular invasion, which makes diagnosis more difficult [7]. Hector Battifora Mesothelium (HBME-l) immunoreactivity was not observed in normalfollicular epithelium, but was seen in scattered histiocytes. HBME-1 expression in various thyroid neoplasms originating from follicular cells[8].

Gal-3 plays an essential role in adhesion of cell to cell and cell to matrix interaction. Galectins are a structurally related group of proteins, defined by having at least one characteristic carbohydrate recognition domain with an affinity for galactosides [9].

Materials and Methods

Study was done in the period between (2015-2017) in biology department in college of Education for pure science /Ibn Al-Haitham at Baghdad University and in pathology department /college of medicine at Al-Nahrain University.

Paraffin blocks of thyroid tissues samples used in this study were collected from laboratories of Baghdad Teaching Hospital, Al-Khadhmiya Teaching Hospital, Al-Yarmouk Teaching Hospital, Al-Kindi Teaching Hospital, Al-Karama Teaching Hospital, Ghazi Al-Hariri Hospital for surgical specialist in Baghdad, Al-Hussein Hospital (Kerbala Health Office) in Karbala, Al-Sadder Medical City in Al-Najaf , Al-Sadder Teaching Hospital(Al-Ashraf/pathology unit) in Basra, Rizgary Teaching Hospital in Erbil, Kalar Educational Hospital in Al- Sulaymaniya and private laboratories, for the years (2006-2016).

The clinicopathological parameters were obtained from patients’ admission case sheets and pathology reports, including age and gender. The study was retrospectively designed. A total of 120 paraffin block were included in the study, 30 blocks were (FC), 30 blocks were (FVPC). 30 blocks were (FA), 30 blocks were blocks thyroid follicular hyperplasia.20 blocks endocervical epithelium.20 paraffin blocks of colonic epithelium.

The clinicopathological parameters were obtained from patients’ admission case sheets and pathology reports (age and gender). From each paraffin block, 4 slides, each of thicken were taken, one was stained with Hematoxylin and Eosin (H&E) for revision of histopathological diagnosis.

The Immunohistochemistry Study (IHC)

Immunohistochemistry (IHC) technique is used for the detection of a specific antibody bound to an antigen in tissue sections. The following steps were undertaken [10]: The histological blocks were cut 5μm thickness. The slides were placed in hot air oven at for 60 minutes 65° C. Deparaffinizing histological sections were inundated successively in the accompanying solutions. Rehydrated the histological sections in descending concentration of ethyl alcohol. Antigen retrieval: there was mild technical modification that was necessarily done to obtain best result for this antigen presentation.

The slides were withdrawn from the antigen retrieval solution. Pap pen was used to draw a circle around the tissue sections. Then Peroxidase block, Diluted primary antibody, Secondary antibody, Hoarse reddish peroxidase (HRP) conjugate, Substrate-chromogen solution, The slides were immersed in a bath containing hematoxylin, The sections were dehydrated in an ascending concentration of ethyl alcohol and Two drops of DPX were applied to the tissue sections.
The Positive Reaction of HBME-1

HBME-1 is strongly expressed in endocervical epithelium; variably positive or focal positive in endometrial glands with luminal (apical) staining pattern and scattered histiocytes in lymphoid tissue. Follicular cells in the thyroid gland. Staining is brown cytoplasmic with membrane accentuation. Technical negative control was obtained by omission of primary antibody [11].

The Positive Reaction of Galectin-3

Galectin-3 is expressed strongly in the colon in a large number of tissues and cell types such as epithelial cells, fibroblasts, keratinocytes, monocytes and macrophages. It is localized in the nucleus, cytoplasm and at the cell surface staining brown.

Scoring of Immunohistochemical Staining

The results of immunohistochemical expression of HBME-1 and Galectin-3 positivity in each individual specimen were analyzed in a semi-quantitative mode as the following:

Galectin-3

Results were expressed in to the percentage of positive tumor cells [12]:
Score 0: No staining or staining is less 10% of tumor cells
Score 1: Staining in 10% to 20% of cells.
Score 2: Staining in 26% to 50% of cells
Score 3: Staining in 51% to 75% of cells.
Score 4: Staining in more than 75% of cells.

Microscopic Examination

The slides for thyroid glands immunohistochemical study have been examined using light microscope (Genax, USA) with 4x, 10, 40x). The sections were examined and then the sections were selected for imaging by light microscope with camera eye piece.

Results

Immunohistochemical Expression of Galectin-3 and HBME-1 Markers in Study Groups

Table (1) and Figure (6) showed the descriptive measures and analysis regarding immunohistochemical expression of Galectin-3 (Gal-3). Mean immunohistochemical score of Galectin-3 expression was 3.06 ±0.68, 2.67±0.50, 1.44 ±0.53 and 0.40 ±0.25 in follicular carcinoma, follicular variant of papillary carcinoma, follicular variant of papillary carcinoma, follicular adenoma and follicular hyperplasia, respectively.

Overall all, there was highly significant difference (P<0.001). Individually speaking, there was no significant difference (P>0.05); in mean score of immunohistochemical score of Galectin-3 between follicular carcinoma and follicular variant of papillary carcinoma however, carcinoma of both types showed significantly higher (P<0.001). Galectin-3 score than both follicular adenoma and follicular hyperplasia In addition, the score of follicular adenoma was significantly greater (P<0.05), than that of follicular hyperplasia as shown in Figure (7).

Frequency of cases with positive expression of Gal-3 is shown in Table (2). The frequency was highest in both follicular carcinoma and follicular variant of papillary carcinoma 100.0%, whereas it was 23.3 % in follicular adenoma and 0% in follicular hyperplasia.

Table 1: Mean Score of immunohistochemical expression of Galectin3 marker in the cases of study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases of Study Groups</th>
<th>Follicular Carcinoma</th>
<th>Follicular Variant of Papillary Carcinoma</th>
<th>Follicular Adenoma</th>
<th>Follicular Hyperplasia</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>3.06 ±0.68</td>
<td>2.67±0.50</td>
<td>1.44 ±0.53</td>
<td>0.40 ±0.25</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>3.00 (0.75)</td>
<td>3.00 (1.00)</td>
<td>1.00 (1.00)</td>
<td>0.00 (1.00)</td>
<td>HS</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.00 -4.00</td>
<td>2.00 -3.00</td>
<td>1.00 -2.00</td>
<td>0.00 -1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Kruskal Wallis test; HS: highly significant; IQR: interquartile range

Table 2: Number and frequency of cases with positive Galectin-3 immunohistochemical expression in the study groups

<table>
<thead>
<tr>
<th>Cases of Study Groups</th>
<th>n (%)Expression of Gal-3 marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Carcinoma</td>
<td>30 (100.0%)</td>
</tr>
<tr>
<td>Follicular variant of Papillary Carcinoma</td>
<td>30 (100.0%)</td>
</tr>
<tr>
<td>Follicular Adenoma</td>
<td>7 (23.3 %)</td>
</tr>
<tr>
<td>Follicular Hyperplasia</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
</tr>
</tbody>
</table>
Figure 1: Positive immunohistochemical expression of Galectin-3 (cytoplasmic brown color; black arrow) in thyroid follicular carcinoma of score 3 (51% to 75% of cells stained; yellow arrow) (IHC, 40X)

Figure 2: Positive immunohistochemical expression of Galectin-3 (cytoplasmic brown color; black arrow) in thyroid follicular carcinoma of score 4 (more than 75% of cells of cells stained; yellow arrow) (IHC, 20X)

Figure 3: Positive immunohistochemical expression of Galectin -3 (cytoplasmic brown color; black arrow) in follicular variant of papillary carcinoma of score 3 (51% to 75% of cells of stained; yellow arrow) (IHC,40X)

Figure 4: Positive immunohistochemical expression of Galectin-3 (cytoplasmic brown color; black arrow) in follicular variant of papillary carcinoma of score 4 (51% to 75% of cells stained; yellow arrow) (IHC, 4X)
Figure 5: Positive immunohistochemical expression of Galectine-3 (cytoplasmic brown color; black arrow) in thyroid follicular adenoma of score 2 (26% to 50% of cells stained) (IHC, A: 10X, B: 40X, C: 10X and D: 40X).

Figure 6: Positive immunohistochemical expression of Galectine-3 (cytoplasmic brown color; black arrow) in thyroid follicular hyperplasia of score 0 (no cells stained) (IHC, 10X).

Figure 7: Positive immunohistochemical expression of Galectine-3 marker (cytoplasmic brown color; black arrow) in thyroid follicular hyperplasia of score 1 (10% to 20% of cells stained) (IHC, A10X: B20X).

Figure 8: Mean Galectin-3 immunohistochemical expression score in the cases of study groups.
Table (3) and Figure (9-17) showed the descriptive measures and analysis regarding immunohistochemical expression of HBME-1. Mean score of immunohistochemical HBME-1 was 3.50±0.52, 3.56 ±0.53, 1.44 ±0.53, 0.20 ±0.15 and in follicular carcinoma, follicular variant of papillary carcinoma, follicular adenoma and follicular hyperplasia, respectively. Overall all, there was highly significant difference (P<0.001). Individually speaking, there was no significant difference (P>0.05) in mean score between follicular carcinoma and follicular variant of papillary carcinoma; however, carcinoma of both types showed significantly higher (P<0.001) of mean score of expression of HBME-1 marker than both follicular adenoma and follicular hyperplasia. In addition, the mean score of follicular adenoma was significantly greater than (P<0.05) that of follicular hyperplasia as shown in Figure (18). Frequency of cases with positive expression of HBME-1 marker is shown in Table (4). It was highest in both follicular carcinoma and follicular variant of papillary carcinoma 100.0%, whereas it was 60.0 % in follicular adenoma and 0% in follicular hyperplasia.

Table 3: Mean score of immunohistochemical expression of HBME1 in the study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases of Study Groups</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular Carcinoma</td>
<td>Follicular Variant of Papillary Carcinoma</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>3.50±052.</td>
<td>3.56 ±0.53</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1.50(1.00)</td>
<td>4.00 (1.00)</td>
</tr>
<tr>
<td>Range</td>
<td>3.00 -4.00</td>
<td>3.00 -4.00</td>
</tr>
</tbody>
</table>

*Kruskal Wallis test; HS: highly significant; IQR: interquartile range

Table 4-8: Number and frequency of cases with positive HBME-1 immunohistochemical expression in the study groups

<table>
<thead>
<tr>
<th>Cases in Study Groups</th>
<th>Expression of HBME-1 Marker n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Carcinoma</td>
<td>30 (100.0%)</td>
</tr>
<tr>
<td>Follicular Variant of Papillary Carcinoma</td>
<td>30 (100.0%)</td>
</tr>
<tr>
<td>Follicular Adenoma</td>
<td>18 (60.0 %)</td>
</tr>
<tr>
<td>Follicular Hyperplasia</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
</tr>
</tbody>
</table>

Figure 9: Positive immunohistochemical expression of HBME-1 (cytoplasmic brown color; black arrow) in thyroid follicular carcinoma of score 3 (51% to 75% of cells stained) (IHC, 40X)

Figure 10: Positive immunohistochemical expression of HBME-1 marker (cytoplasmic brown color; black arrow) in thyroid follicular carcinoma of score 4 (more than 75% of cells) (IHC, 40X)
Figure 11: positive immunohistochemical expression of HBME-1 marker (cytoplasmic brown color; black arrow) follicular variant of papillary carcinoma score (51% to 75% of cells stained) (IHC, 20x)

Figure 12: Positive immunohistochemical expression of HBME-1 marker (cytoplasmic brown color; black arrow) follicular variant of papillary carcinoma of score 3 (51% to 75% of cells stained) (IHC, 10x)

Figure 13: Positive immunohistochemical expression of HBME-1 (cytoplasmic brown color; black arrow) in thyroid follicular adenoma of score 2 (26 to 50% of cells stained) (IHC, 10X)

Figure 14: Positive immunohistochemical expression of HBME-1 marker (cytoplasmic brown color; black arrow) in thyroid follicular adenoma of score 1 (10% to 20% of cells stained; yellow arrow) (IHC, 20X)
Figure 15: Positive immunohistochemical expression of HBME-1 marker (cytoplasmic brown color; black arrow) in thyroid follicular adenoma of score 1 (10% to 20% of cells stained) (IHC, 40X)

Figure 16: Positive immunohistochemical expression of HBME-1 marker (cytoplasmic brown color; black arrow) in thyroid follicular hyperplasia of score 1 (10% to 20% of cells stained) (IHC, 10X)

Figure 17: Positive immunohistochemical expression of HBME-1 marker (cytoplasmic brown color; black arrow) in thyroid follicular hyperplasia of score 1 (10% to 20% of cells stained) (IHC, 10X)

Figure 18: Mean HBME-1 immunochemical expression score in the study groups
Discussion

The mean score of immunohistochemical expression of Galectin-3 (Gal-3) was proved, in this study, to be significantly higher in thyroid nodules exhibiting malignant behavior follicular carcinoma and follicular variant of papillary carcinoma than those nodules carrying benign and or reactive lesions (follicular adenoma and follicular hyperplasia), moreover, the mean expression of Gal-3 was significantly highest in follicular carcinoma group. These results suggest that Gal-3 immunohistochemical expression can be used as an adjunct modality to aid in the diagnosis of thyroid malignancy. These results are consistent with a lot of published literatures [13, 14, 15, 16].

Galectins are a family of animal lectins with diverse biological activities. They function both extracellularly, by interacting with cell-surface and extracellular matrix glycoproteins and glycolipids, and intracellularly, by interacting with cytoplasmic and nuclear proteins to modulate signalling pathways. Current research indicates that galectins have important roles in cancer; they contribute to neoplastic transformation, tumor cell survival (anti-apoptotic role), angiogenesis and tumor metastasis. They can modulate the immune and inflammatory responses and might have a key role helping tumors to escape immune surveillance [17, 18].

Galectin-3 is a β-galactoside-binding lectin. It cross-links glycoproteins at the cell surface forming a lattice that inhibits endocytosis of epidermal growth factor receptor (EGFR), thereby enhancing its function. Cytoplasmic location of Galectin-3 is related to anti-apoptotic function, induced by abnormal p53 expression.

Therefore, the cytoplasm/membrane location in thyroid follicular, rather than nuclear presentation, of Galectin-3 is for malignancy [19]. The possible explanation for the higher immunohistochemical level of Galectin-3 in follicular carcinoma (FC) and follicular variant of papillary carcinoma (FVPC) than in follicular adenoma is probably due to a genetic mutation that favors cytoplasmic and membrane localization of Galectin-3 protein rather than nuclear localization [20]. In the current study, carcinoma of both types follicular thyroid carcinoma (FTC) and follicular variant of papillary carcinoma (FVPC) showed significantly higher HBME-1 score than both follicular adenoma and follicular hyperplasia (P<0.001). This means that HBME-1 is a reliable marker for differentiating malignant thyroid lesions from benign ones.

This finding is in concordance with a lot of published articles [14, 15, 21, 22, 23, 24]. Moreover, we were able to address a cutoff value for the immunohistochemical expression of HBME-1 score of > 2 to segregate malignant from benign thyroid nodule with an area under the curve of 0.980 (95% CI: 0.936-0.997), sensitivity of 90 % and specificity of 98.33%. [25] Have worked a lot of immunohistochemical markers in 51 papillary thyroid carcinoma (PTC) and 57 benign thyroid lesions. They have found HBME-1 staining in 96% of the malignant group and staining was not observed in 93% of benign lesions.

This finding is approximately similar to the finding of the current study in which 100% of cases with follicular carcinoma were positive for HBME-1; however, 60 % of cases with follicular adenoma expressed this marker and this is far more than that reported by [25]. In another study, 72.3% of PTC group had diffuse and strong staining with HBME-1 marker, and the staining was not observed in 92.3% of benign lesions; there is a significant difference between PTC group and benign group for the HBME-1 staining (P < 0.01) [23].

These findings agree with present study regarding follicular variant of papillary carcinoma but not with follicular adenoma. The explanation for the finding that benign lesions in the previous two studies [23] and [25], may be attributable to the inclusion of hyperplastic nodules with the benign lesions, it has been proven by the present study that all cases with follicular hyperplasia were negative to HBME-1. HBME-1 is a component of the microvilli on the surface of mesothelial cells and has been used for the diagnosis of tumors that originate from mesothelial cells. Previous studies [14, 26, 27], demonstrated that a high rate of HBME-1-positivity, by Immunohistochemistry was observed in malignant thyroid tissues, and HBME-1 was a sensitive marker for papillary
thyroid carcinoma. In another study, when assessing the diagnostic performance of HBME-1 as a single protein marker, the sensitivity was 85.3% [12]. The results of the present are approximately similar to that of [28] who stated, following performance of ROC analysis, that HBME-1 was found to have high sensitivity 94.5 % and specificity 77.08 % for papillary thyroid carcinoma diagnosis.

References


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