Detection of Some Biomarkers in Iraqi Patients with Thyroid Carcinoma

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Abstract:
Background: Thyroid cancer, the most common endocrine malignancy, has increased dramatically in the last years. Cathepsin D and Galectin-3 have been implicated in the regulation of cellular growth, differentiation, and malignant transformation in various organs including thyroid gland. It has been extensively studied as an immunohistochemical (IHC) marker of these neoplasms in thyroid malignancy.

Aim: To assess the diagnostic role of galectin-3 (Gal-3) and cathepsin D (CD) in thyroid neoplasm.

Materials and Methods: In this study we evaluated Cathepsin D and Galectin-3 expression in 43 of thyroid tissue cases, (23) thyroid malignant, (12) benign and (8) control cases. The final common diagnosis arrived at after histopathological evaluation of H&E stained sections by pathologist was considered the final H and E standard diagnosis. The malignant neoplasms included Papillary Thyroid Carcinoma (PTC), Follicular Thyroid Carcinoma (FTC), Medullary Thyroid Carcinoma (MTC), Anaplastic Thyroid Carcinoma (ATC) and Hürthle cell cancer (HCC). The benign neoplasms included Follicular Adenoma (FA). IHC staining for CD and Gal-3 was performed for all cohort.

Statistical Analysis: Statistical analysis was done by using Pearson and Spearman correlations to calculate p-value to statistical significance.

Results: CD and Gal-3 expression were significantly higher in malignant thyroid neoplasms as compared to benign neoplasms (p<0.01). As well highly significant correlation of expression between galectin-3 and cathepsin D (p<0.01).

Conclusion: Diffuse and strong IHC staining for galectin-3 and cathepsin D in malignant, very weak stain score 1 inbenign tumour and no stain in control. These markers can be used in the diagnosis of thyroid neoplasms with equivocal morphologic features.

Keywords: Thyroid cancer, IHC, cathepsin D, Galectin-3.

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I. Introduction

Thyroid carcinoma is the most common endocrine malignancy and it comprises 3% of all newly diagnosed malignancies (Jemalet al., 2010), follicular carcinoma represents more than 90% of thyroid carcinoma, the papillary thyroid carcinoma (PTC) accounts for a majority of these new diagnoses, other types includes follicular thyroid cancer (FTC, up to 11%), Hürthle cell cancer (HCC, 3%) and anaplastic thyroid cancer (ATC, 2%), PTC and FTC are commonly referred to together as differentiated thyroid cancer (DTC), while ATC represents undifferentiated thyroid cancer. The other type of thyroid carcinoma that originated form non-follicular thyroid cell (calcitonin producing cells) called medullary thyroid carcinoma which accounts 4% of thyroid cancers (Brown et al., 2011). In addition to morphology, much attention has been paid to the prognostic, predictive, and therapeutic potential. In this regard, the diagnostic approach to these tumors should include IHC markers that can aid in better assessment of morphologic details (Fisher and Asa, 2008).

Galectins are a family of lectins that are predominantly localized in the cytoplasm, translocate to the perinuclear membrane, nucleus and/or get secreted from the cytoplasm (Aiad et al., 2008; Cvejic et al., 2005; Nangia-Makker, 2007). Galectin-3 is participates in cellular functions such as cell proliferation, apoptosis, cellular transformation, tumour progression and metastasis of cancer cells. In the recent times Galectin-3 has received notable recognition for its usefulness as a diagnostic marker for thyroid cancer (Chiu et al., 2010), the utility assessed by studies have reported encouraging results (Webber et al., 2004; Hermann et al., 2002).

Cathepsin D (CD) is an aspartic lysosomalendopeptidase, it is present in almost cells and tissues of mammals. Overexpression of this protease has been related with the progression of several human cancers such as thyroid carcinomas, squamous cell carcinoma, renal cell, carcinoma, glioma brain tumors, laryngeal...
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carcinoma, breast cancer, lung cancer, ovarian carcinoma (Jacobson-Raberet al., 2011, Tan et al., 2013). Cathepsin D play an important role in cancer, it is promote tumor growth directly by acting to degrade and remodel the basement membrane and interstitial stroma surrounding the primary tumor and indirectly by activation of other enzymes or in collaboration with other cathepsins in the proteolysis process (Derochet al., 2012). The levels of cathepsin D in tumors were documented to be higher than in adjacent normal tissue (Szaajda et al., 2008). In this study we aim to evaluate the expression of cathepsin D and galectin-3 in thyroid carcinoma.

II. Materials And Methods

A retrospective study of 43 paraffin blocks of thyroid tissue cases were selected from archive files of Histopathology department in the Central Public Health Laboratory, Baghdad, Iraq, H and E stained sections were re-examined by consultant pathologist and the final diagnosis of the tumors types in accordance with World Health Organization classification. These cases included 23 cases were malignant, 12 benign and 8 cases were control.

Immunohistochemistry

Immunohistochemical evaluation of galectin-3 and cathepsin D were performed on representative histologic sections of the thyroid neoplasms. Sections were de-waxing in xylene and rehydrated through ethanol. Antigen retrieval was done with Tri-sodium citrate buffer (pH 6.0 to 6.2) using microwave oven. Slides were brought to room temperature. Slides were incubated in peroxidase – blocking solution (Dako, ready-to-use) for 20 min. Non-specific binding of antibodies was blocked by the addition of 2.5% normal horse serum, from (ImmPRESS™ Vector, USA). Primary antibody for cathepsin D was diluted (1:1000) and for Gal-3 was diluted (1:200) using antibody diluant (ready-to-use, Code No.ab64211Abcam, Cambridge, UK), and incubated for 1 hour at room temperature. After that secondary antibodies (Anti Rabbit. peroxidase, Cat. No. MP-7401, ImmPRESS™ Vector, USA) for cathepsin D and (anti mouse peroxidase, Cat. No. MP-7402, ImmPRESS™ Vector, USA) for galectin-3 were applied to the slides and incubated for 30 minutes at room temperature in a humidified chamber. The colorimetric detection of reaction was achieved by the diaminobenzidine (DAB) Peroxidase Substrate method. Then, sections were counterstained with haematoxylin, dehydrated, and mounted.

Immunohistochemical Scoring System

Gaelectin-3

Scoring for all the immune expression results were assessed with the aid of a specialist pathologist. Positive galectin-3 staining was considered when any cytoplasmic brown staining was seen according to scoring system submitted by Weber et al. (2004) and Hermann et al. (2002). This system was evaluated semiquantitatively by using two parameters evaluated; percentage of tumor cell stained and the intensity of stain. The staining intensity score (IS) was graded on a scale of 0 to 3 where 0, 1+, 2+, and 3+ denote no staining, weak/slight staining, moderate staining and intense staining respectively, and the proportion score (PS) of stained cells were interpreted as 1+ (<5% of cells), 2+ (5% to 50% of cells) and 3+ (>50% of cells). Positive result denotes the specific staining of more than 5% of the tumour cells with slight, moderate or intense staining.

Cathepsin D

The positive cells was scored semiquantitatively according to Allgayer et al. (1997), this system depend on the number of positive stain tumor cells; Score 0: negative; Score 1: 30% positive tumor cells; Score 2: 30–70% positive tumor cells; Score 3: 70% positive tumor cells.

Statistical Analysis

Statistical analysis was performed using SPSS 20. Descriptive statistic for age and sex, Spearman and Pearson correlations for ordinal variables to determine correlations of biomarkers with histological type as well between expressions of two markers. A P value of <0.01 was considered as significant.

III. Results

Thirty five of Iraqi patients with thyroid tumors were studied. Patient's age ranged from (20-75) years with a mean of 36.91±11.17 years. Females constituted 26 (61%) while male 17 (39%) of the cases. The malignant neoplasms were more common were 23 (64%), 12, 5, 4, and 1 were PTC, MTC, FTC, ATC and HCC respectively, while benign were 12 (28%). Eight samples were used as control cases 8 (19%) (figures 1 and 2). This study demonstrated positive immunostaining of cathepsin D and galectin-3 in cytoplasm of cancer cells as a brown diffuse pigmentation (Figure-3). Results of IHC expression of cathepsin D and galectin-3 in thyroid tissue specimens are summarized in table 1.
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Table 1. Immunohistochemical expression of galectin-3 and cathepsin D according to histological classification.

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>Total</th>
<th>Galectin-3</th>
<th></th>
<th></th>
<th>Cathepsin D</th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PTC</td>
<td>12</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>FTC</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MTC</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ATC</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HCC</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Benign</td>
<td>12</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
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**Figure 3.**
(A) ATC, IHC of CD, score 3 200x, (B) ATC, IHC of Gal3, score 3 200x, (c) PTC, IHC of CD, score 2 200x, (d) PTC, IHC of Gal3, score 2 200x, (e) MTC, IHC of CD, score 3 200x, (f) MTC, IHC of Gal3, score 3 200x, (g) FTC of CD, score 3 200x, (h) FTC of Gal3, score 2 200x (i). PTC, IHC of Gal3 score 3 200x.

**Table 2. Association of histological type with expression of cathepsin D**

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>No. of Cases</th>
<th>IHC Expression of Cathepsin D</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>23 (53%)</td>
<td>Positive 17; Negative 6</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Benign</td>
<td>12 (28%)</td>
<td>Positive 7; Negative 5</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8 (19%)</td>
<td>Positive 0; Negative 8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43 (100%)</td>
<td>Positive 24; Negative 19</td>
<td></td>
</tr>
</tbody>
</table>

(*p<0.01)

As well as the current study showed that a highly significant relationship between immunohistochemical expression of galectin-3 with histological type p value (0.005). Table 3

**Table 3. Association of histological type with expression of galectin-3**

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>No. of Cases</th>
<th>IHC Expression of Galectin 3</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>23 (53%)</td>
<td>Positive 12; Negative 11</td>
<td>0.005*</td>
</tr>
<tr>
<td>Benign</td>
<td>12 (28%)</td>
<td>Positive 8; Negative 4</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8 (19%)</td>
<td>Positive 0; Negative 8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43 (100%)</td>
<td>Positive 20; Negative 23</td>
<td></td>
</tr>
</tbody>
</table>

(*p<0.01)

**IV. Discussion**

Follicular epithelial cell-derived thyroid cancers are the most common and increasingly incident endocrine malignances, in this study the follicular carcinoma constitutes the vast majority than non-follicular carcinoma, these results were agree with previous study by Brown et al. (2011). PTC wereconstituted the majority of the malignant cases this results agree with (Chen et al., 2009), they reported that the PTC increasing in new diagnoses of thyroid carcinoma. In this study,cathepsin Dand galectin-3 were specifically expressed by malignant neoplasm, showing that these markers could be used as a support biomarker for thyroid cancer. Galectin-3 has important roles in variety biological events, such as differentiation, proliferation, cell adhesion, growth, apoptosis and regulation of immune system. Also galectin-3 is expressed in various cells and plays an important role in tumorgenesis, angiogenesis, tumor metastasis and malignant transformation (Lee et
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Galectin-3, has received significant recent attention for its utility as a diagnostic marker for thyroid cancer represents the well-studied molecular candidate for thyroid cancer diagnosis. The current result showed that the cytoplasmic expression of galectin-3 was found in carcinomas and not found in normal thyroid tissue while a very weak expression in adenoma cases, this study documented a significant correlation between malignant cases with galectin-3 expression, most of authors reported very low galectin-3 positivity in adenomas making this molecule a useful marker in distinguishing thyroid carcinoma and adenomas (Barroeta et al., 2006), similar observation was noted by Mehrotra et al. (2004). Study by Chui et al. (2010), they were found to highly expressed of galectin-3 in thyroid carcinoma, but not expressed in normal thyroid tissue, and very low frequently in benign thyroid cases, so they found that galectin-3 might also represent an attractive target for therapy of thyroid cancer. As well, Baruet al. (2010) were studied the immunohistochemical expression of galectin-3 in the diagnosis and differential diagnosis of malignant and normal thyroid lesions. They suggested that, the use of galectin-3 might provide significant contributions in the differential diagnosis of malignant thyroid tumors. According to current results and previous studies we can conclude that immunohistochemical expression of galectin-3 may be highly related with malignancies and could be used to differentiated malignancies from normal tissues but not differentiated between malignant and benign.

Cathepsin D (CD) has different biological functions, its playing an important in tumor progression such as, proliferation, angiogenesis, invasion and metastasis (Tan et al., 2013; Letoet al., 2004). The current study revealed that the cathepsin D was positive expression in majority of malignant cases confirming its sensitivity as marker for malignancies, 7 out of 12 (58%) of PTC were positive, all four cases of FTC were positive, 4 out of 5 (80%) of MTC were sensitive for cathepsin D. Hürthle cell cancer was positive for cathepsin D expression, the benign cases were low weak and negative expression, while negative expression in control tissues, this results agreed with (Tan et al., 2013), they were found elevated of cathepsin D in thyroid carcinoma and other types of solid tumors. The current results found highly significant correlation between cathepsin D expression with malignant tumors. These results closed with study of (Szajda et al., 2008), they reported that the levels of cathepsin D in tumors were higher than in adjacent normal tissue. From these findings we can conclude the role of cathepsin D in the progression of this tumors comparison with normal tissue and adenoma cases. It can be suggested that the cathepsin D considered a diagnostic marker for thyroid lesions because it specific for malignancies. As well, the current result found high significant association between immunohistochemical expression of cathepsin D and galectin-3, no previous study was assessed this association but we can be concluded form these results that the cathepsin D and galectin-3 play an important role in tumor progression. Experimental and clinical data showed a correlation between galectin-3 expression and tumor progression and metastasis (Balan et al., 2010), as well cathepsin D is submit to assist early stages of tumorgenesis such as cell proliferation and local invasion and metastasis (Tan et al., 2013). From above we can explain this highly association because these two makers were expressed in proliferation, invasion and distal metastasis of thyroid tumor cells.

V. Conclusion

In conclusion, cathepsin D and galectin-3 are good biomarkers of malignancies especially in supporting the diagnosis of thyroid carcinoma. If thyroid carcinomas can give negative expression for both (cathepsin D and galectin-3) or for one of them (cathepsin D and galectin-3), hence we cannot depend on this negative biomarker expression as a diagnostic method to detect thyroid malignancy. Finally, this current result can be recommended to add cathepsin D and galectin-3 to routinely diagnosis markers in our land for detecting of thyroid malignancies cases.

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References


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