Evaluation of Galectin-3, β-catenin and nuclear morphometry in different thyroid lesions

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Abstract
PURPOSE: This study aimed to evaluate the expression pattern of Galectin-3 and β-catenin and to assess the nuclear morphometric features of different thyroid lesions to investigate their diagnostic significance.

METHODS: Sixty specimens of benign and malignant thyroid lesions, in addition to 10 cases of normal thyroid tissue used as control, were immunohistochemically stained with antibodies against galectin-3 and β-catenin. Hematoxylin and Eosin stained slides were examined using image analyzer Proplus V software. Morphological parameters measured included mean nuclear diameter, mean nuclear perimeter and mean nuclear area.

RESULTS: Cytoplasmic immunoreactivity for galectin-3 was strongest in papillary thyroid carcinomas (88.9%), whereas staining was less intense in follicular variant of papillary thyroid carcinoma and in follicular (60% and 75% respectively) and poorly differentiated carcinomas (28.6%), and expressed focally and weakly in follicular adenoma cases and even weaker in the reactive follicular epithelium of nodular goiter (P < 0.5). Membrane β-catenin expression was decreased in only two out of 13 (15.4%) adenomas and in 21 out of 38 (55.3%) of carcinomas (P < 0.5). Among carcinomas, reduced membrane β-catenin was associated with progressive loss of tumor differentiation and increased invasiveness (P < 0.5). Mean nuclear diameter and the mean nuclear perimeter were higher in undifferentiated carcinomas when compared to other subtypes and were the least for follicular neoplasms.

CONCLUSIONS: Galectin-3 immunostaining and analysis of β-catenin dysregulation may be useful as an adjunct to distinguish benign from malignant thyroid lesions. Quantitative estimation of nuclear features can play a role in assessment of the morphological features and so helping in diagnosis of thyroid lesions.

Introduction

Thyroid cancer is a common endocrine malignancy with an apparent increasing incidence. A survey sponsored by the World Health Organization (WHO) in 2010 revealed that there are around 44,670 new cases and 1,690 deaths caused by this disease every year (Jemal, et al; 2010). Among thyroid malignancies, papillary thyroid carcinoma (PTC) is the most common

According to Egyptian National Cancer Institute, malignant thyroid gland tumors constituted 65.31% of endocrine malignant tumors and the most common tumor was papillary thyroid carcinoma (PTC) which constitutes 67.59% (Mokhtar et al., 2007).

For uncertain reasons, the incidence of thyroid cancer appears to be rising, although the outcome remains excellent with long-term disease-free survival rates. Appropriate clinical management and prognosis largely depends on the diagnostic reliability of histopathological examination on the surgically-removed thyroid tissue (Ringel and Ladenson, 2004).

However even with application of the diagnostic criteria, such as characteristic nuclear appearances in PTC, distinguishing it from thyroid papillary hyperplasia is extremely challenging due to tumor heterogeneity. Occasionally, cases of papillary thyroid hyperplasia, in particular solitary nodules with papillary change, can simulate papillary thyroid carcinoma and cause a diagnostic dilemma (Casey, et al; 2003).

In addition interobserver variations still lead to low diagnostic reproducibility, especially in the diagnosis of follicular carcinoma (FCA) (Hirokawa et al; 2002, Franc et al; 2003, de Matos et al; 2012, Papale et al; 2013). Also, the morphologic distinction between benign and malignant thyroid follicular lesions can sometimes be challenging, therefore an immunohistochemical marker to aid in this distinction would be useful (Matsuo, et al; 2004).

Galectins are structurally-related proteins of the lectin family, defined by having at least one characteristic carbohydrate recognition domain with an affinity for β-galactosides (Barondes, et al; 1994, Lee and Lee; 2013).

Fourteen different galectins have been characterized. Due to the potential of galectins to participate in cell-cell and cell-matrix adhesion, growth regulation and internal processes, it is deduced that this galectin family should be involved in pathological expression (Perillo, et al; 1998, Hughes; 2001, Lee and Lee; 2013).

Among various galectins, Gal-1, Gal-3 and Gal-7 are of interest in thyroid malignancy (Danguy, et al; 2002). It was mentioned that a combination of Gal-7 expression together with Cytokeratin-19 markers have some important diagnostic value in distinguishing the encapsulated follicular variant of PTC from microfollicular adenomas (Rorive, et al; 2002). Galectin-3 is a galactoside-binding protein that is expressed in many cell types (Liu and Rabinovich; 2005) and is found inside cells, extracellularly (but still cell
surface-associated) and in the circulation. Intracellular galectin-3 is an apoptosis inhibitor and mRNA splicing promoter whilst extracellular cell surface-associated galectin-3 acts as an adhesion molecule during cell-cell interactions and is associated with metastasis (Takenaka, et al; 2004, Liu and Rabinovich; 2005).

βeta-Catenin is part of a membrane-bound cell growth-signaling complex that plays a role in cell adhesion, as well as in promotion of growth through activation of the Wnt signaling pathway. Oncogenic signaling occurs when beta-catenin is released, accumulates in the cytoplasm, translocates into the nucleus, and promotes transcription of genes including bel-1 (cyclin D1) and c-myc that induce cell proliferation (Rezk et al., 2004, Giunti, et al; 2013).

β-catenin dysregulation has been shown to play an important role in human tumorigenesis (Peifer and Polakis; 2000, Romitti, et al; 2013). Activating β-catenin mutations were discovered in a variety of human neoplasms ranging from colonic adenocarcinoma, where they are present in approximately half of the tumors with wild-type APC (Sparks, et al; 1998), to malignant fibrous histiocytoma (Miller, et al; 1999). Although a reduction of β-catenin bound to the cell surface has been demonstrated in thyroid carcinoma (Garcia-Rostanm et al; 1999, Huang, et al; 1999), the biological and clinical relevance of β-catenin dysregulation in thyroid neoplasia is primarily unknown.

Computerized morphometry is a scientific tool to evaluate cellular changes enhancing the interpretation of morphological features by the transformation of pathological changes in cells to a qualitative form (Sheng-Lan, et al, 2005). Nuclear morphometric features of malignant cells differ from those observed in non-malignant cell nuclei. This observation led to the hypothesis that such changes occur prior to the emergence of clinically detectable disease and that nuclear morphometry can be used as a biomarker for estimating an individual’s risk for cancer (Boone, et al, 2000).

Morphometry may complement cytological diagnosis and provide useful information. The potential significance of this technique is to distinguish between benign, borderline and malignant lesions, for objective grading of invasive tumors, prediction of prognosis and therapeutic response (Priya and Sundaram, 2011). Morphometry has been described for more than a century because the histological characteristics of normal and abnormal cells have been used as a measure of prognosis and as a way of predicting the cause of the disease (Shih, et al; 2013). The usefulness of quantitative morphometric analysis has helped in cytological grading of breast lesions, but has not yet received widespread acceptance in thyroid lesions owing to limited references and subjectivity (Baloch et al., 2002).

The aim of this study was to examine the expression patterns of galectin-3 and β-catenin as well as to assess the nuclear morphometric features
in different thyroid lesions, to clarify their roles in diagnosis and to explore their correlations with clinicopathological parameters.

Material and Methods:

Material: A retrospective, controlled study comprised formalin-fixed, paraffin-embedded blocks of tissues from patients with thyroid lesions were retrieved from the archives of the Departments of Pathology, National Cancer Institute (NCI), Cairo University and Benha Faculty of Medicine, Benha University. The patients were unknown to us as there was no direct contact with them. Tissue blocks with sufficient thyroid tissue, including capsular components, were selected. A total of 60 specimens of benign and malignant thyroid lesions, in addition to 10 cases of normal thyroid tissue adjacent to lesional tissue used as control group. All patients had undergone surgical removal of thyroid lesions between the years 2007 and 2011.

Histopathological Study: From each block serial sections were prepared. Histological sections, four microns thick, were stained by Hematoxylin and Eosin (H&E) for histopathological and nuclear morphometric study of different thyroid lesions. The studied H&E sections were reviewed and were also used to select representative areas of the tumor for subsequent immunohistochemical study.

Nuclear morphometry Study:
Morphometric analysis of cases studied was carried out by means of Olympus soft imaging system, Analysis lifescience program, to measure the nuclear area, the minimum diameters (Min D) and maximum diameters (Max D) of the examined nuclei of thyroid carcinoma cases with an optical magnification x400 on routine H&E stained sections.

According to this method, 60 tumor cell nuclei - in randomly chosen fields within a well-preserved and highly cellular area of the tumor - were selected for each case. The mean nuclear area (MNA) i.e. cross sectional area for each case was determined in square microns (µm2). Both minimum and maximum diameters were used to calculate the mean nuclear elongation factor (MNEF) (min D/ Max D). The elongation factor expresses the degree of nuclear ellipticity. The value of 1.0 represents a circle and <1.0 an elliptical structure (Bakr, et al, 2000 and Nativ, et al, 2001). The ratio of MaxD/MinD (LD/SD) was also calculated (Kefeli, et al, 2010).

Immunostaining:

From each paraffin block 4 µm thick sections mounted onto postive charged slides were taken for immunohistochemical staining. The monoclonal antibodies (for Galactin-3 and B-catenin) were used. Dilution and incubation times are listed in table 1. Streptavidin-biotin immunoperoxidase method was used (Dako, Universal LSAB_2 kit). 3, 3’ diaminobenzidine tetrahydrochloride
(DAB) solution was used as the final chromogen, and sections were counterstained with Mayer’s hematoxylin before mounting.

Negative controls for non-specific binding; incubated with secondary antibodies only; were processed and revealed no signals. Positive controls (breast tissue for galactin-3 and for B-catenin) *(Castronovo et al., 1996)* recommended by manufacturer were used to confirm correct immunohistochemical staining. Slides were screened and observed by two pathologists, who had no prior access to the H&E report of the specimens by using code numbers for each block, to avoid bias. Morphology and cytological appearances were recorded. Scoring was done based on the intensity of staining characteristics on a scale of 1 to 3; a score of 1 indicates focal/weak staining, a score of 2 indicates moderate staining, and a score of 3 for strongly positive staining reaction. The mean of the two scores was calculated. Data analysis was performed with the Statistical Package for Social Sciences version 12.0.1 (SPSS Inc, Chicago, IL, USA). Descriptive analysis of the variables and statistical significance of the tests were expressed in p-value.

**Table 1: Characteristics of antibodies used in this study.**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Incubation time</th>
<th>Source</th>
<th>Staining pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactin-3</td>
<td>Ready to use</td>
<td>2 hrs</td>
<td>Neomarkers</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>B-catenin</td>
<td>1:50</td>
<td>2 hrs</td>
<td>Neomarkers</td>
<td>Membranous, Nuclear or Cytoplasmic</td>
</tr>
</tbody>
</table>

**Immuostaining interpretation:**

**Galactin-3** reactivity was classified according to *Collet et al., (2005)*, as follows:
- Score (1): 1-25% of tumor cells showed cytoplasmic staining of galactin-3;
- Score (2): 25 to 50% of tumor cells showed cytoplasmic staining of galactin-3;
- Score (3): over 50% of tumor cells showed cytoplasmic staining of galactin-3.

**β -catenin:**

Immunoreactivity was expressed according to *Garcia-Rostan et al., (2001)* as the percentage of positively stained target cells in each of four intensity categories (0, no staining; 1+, weak but detectable above control; 2+, distinct; 3+, intense). For each tissue section, a numerical value (the H or histology score) was derived by summing the percentages of cells staining at each intensity multiplied by the weighted intensity of staining 15 after random high-power field observations (×400) corresponding to at least 4,000 tumor cells. The intense membranous staining of normal thyroid follicles adjacent to the tumor was used as internal standard for the scoring of β-catenin expression. Membranous, cytoplasmic, and nuclear immunoreactivity were evaluated by
separate H scores but, given the relative paucity of tumor cells with nuclear or cytoplasmic immunostaining and the resulting low H scores, nuclear and cytoplasmic immunoreactivity patterns were simply recorded as positive or negative with no cut-off values for statistical analysis.

Results
The cases studied included 9 papillary thyroid carcinoma (PTC), 10 papillary thyroid carcinoma follicular variant (PTCFV), 12 follicular carcinoma (FCA), 7 poorly differentiated carcinoma (PDCA), 13 follicular adenoma (FA) and 9 nodular goitre (NG). The age in adenoma cases ranged from 34 years up to 74 years, with a mean age of 46.6 years. For malignant cases, the age ranged from 20 years up to 79 years, with a mean age of 53.2 years.

**Galectin-3 immunostaining results:**
Cytoplasmic Gal-3 immunostaining has been found overexpressed in all malignant thyroid neoplasms (Fig 1 and 2). However, it was not or weakly detectable in normal and non-malignant tissue and follicular adenoma. It was expressed in nodules with cytological atypia. The non-malignant hyperplastic papilla stained weakly, compared to malignant papillae in PTC studied which showed strong expression of galactin-3. Galectin-3 was also expressed focally and weakly in reactive follicular epithelium. Immunoreactivity was strongest in papillary thyroid carcinomas (100% had score 2&3), whereas staining was less intense in the follicular variant of papillary thyroid carcinoma (90% had score 2&3), and even weaker in poorly differentiated carcinomas (57.2% had scores 2&3). Staining was weak in three cases (25%) of follicular thyroid carcinoma (table 2). In several tumors, staining was stronger at the advancing invasive edge of the lesion (Fig 3) than in the central portion of the tumor. Also, a characteristic positive staining reaction in the capsular invading cells in FCA was also observed. Gal-3 expression was also observed in fibroblasts, endothelial cells, macrophages, histiocytes, red blood cells and inflammatory infiltrates in the thyroid tissue.
Table (2): relation of Galectin-3 to tumor type, lymph node metastases, capsular and blood vessel invasion

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. of cases</th>
<th>Score of Galactin-3 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 (1-25%)</td>
</tr>
<tr>
<td>NG</td>
<td>9</td>
<td>9(100%)</td>
</tr>
<tr>
<td>FA</td>
<td>13</td>
<td>13(100%)</td>
</tr>
<tr>
<td>FC</td>
<td>12</td>
<td>3(25%)</td>
</tr>
<tr>
<td>PTC</td>
<td>9</td>
<td>0(0%)</td>
</tr>
<tr>
<td>PTCFV</td>
<td>10</td>
<td>1(10%)</td>
</tr>
<tr>
<td>PDTCA</td>
<td>7</td>
<td>4(42.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lymph node metastases</th>
<th>No. of cases</th>
<th>Score of Galactin-3 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>25</td>
<td>8(32%)</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Capsular invasion</th>
<th>No. of cases</th>
<th>Score of Galactin-3 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>13</td>
<td>7(53.8%)</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>1(4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood vessel invasion</th>
<th>No. of cases</th>
<th>Score of Galactin-3 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>17</td>
<td>8(47.1%)</td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>

NG= nodular goitre  
FA= follicular adenoma  
FC= follicular carcinoma  
PTC= papillary thyroid carcinoma  
PTCFV=papillary thyroid carcinoma follicular variant  
PDTCA=poorly differentiated thyroid carcinoma

A statistically significant correlation (p<0.05) was found between galectin score and type of thyroid tumor. All hyperplastic and benign lesions had low score (1), while most of carcinomas showed high expression (score 2 and 3). All cases with positive LN metastases showed high expression of galectin (score 2 and 3), this was statistically significant (p<0.05). Out of 25 cases with positive capsular invasion, 22 cases (76%) showed high expression for galectin (score 2 and 3), another statistically significant correlation (p<0.05).
Out of 21 cases with positive blood vessel invasion, 20 cases (95.2%) showed high expression of galectin (score 2 and 3), which also was a statistically significant correlation (p<0.05)
High Galectin expression is related to presence of thyroid carcinomas, regardless the type of carcinoma. It is also related to presence of LN metastases, capsular and blood vessel invasion (table 2).

**β-Catenin immunostaining results:**
Normal thyroid follicular cells from perilesional tissue showed strong membrane β-catenin immunoreactivity with no nuclear or cytoplasmic localization. This strong membranous staining of normal tissue provided an internal control for staining distribution and intensity. Three patterns of immunoreactivity were observed in the thyroid tumors: 1) membranous (Fig 4), 2) nuclear, and 3) cytoplasmic (Figure 5).
Membrane β-catenin expression was decreased in all of the thyroid carcinomas. Out of all 38 malignant cases, 44.7% showed membranous β-catenin expression compared to 84.6% of 13 adenoma cases which showed membranous expression. However, 55.3% of malignant cases showed nuclear or cytoplasmic expression. Analysis of variance comparison of the means of the raw β-catenin H scores demonstrated a significant difference between follicular adenomas and all carcinomas (P < 0.01) or the well-differentiated follicular carcinoma group (p<0.05). Membrane β-catenin reduction correlated with progressive loss of tumor differentiation (P < 0.01) (Table 3). Nuclear β-catenin expression was observed only in undifferentiated anaplastic carcinomas (4 of 7 cases, 57.1%) indicating that also aberrant nuclear localization of β-catenin is a marker for loss of tumor differentiation (P < 0.01). Nuclear expression was associated with marked reduction or complete loss of membrane immunoreactivity among thyroid carcinomas (P < 0.01, analysis of variance). In other two cases there was focal but distinct cytoplasmic immunoreactivity. There was significant difference in β-catenin expression among different histological types of thyroid carcinoma such as 66.7% of well-differentiated papillary versus 33.3% of follicular carcinoma, showed membranous β-catenin expression (Table 3). Cytoplasmic and/or nuclear expression was significantly associated with invasive tendency of the thyroid carcinoma (vascular invasion, capsular invasion and lymph node metastasis) (p<0.05).
Table (3): relation of β- Catenin expression to tumor type, lymph node metastases, capsular and blood vessel invasion

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. of cases</th>
<th>β- catenin expression</th>
<th>Membranous</th>
<th>Cytoplasmic or nuclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>9</td>
<td>9(100%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>13</td>
<td>11(84.6%)</td>
<td>2 (15.4%)</td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td>12</td>
<td>4(33.3%)</td>
<td>8(66.7%)</td>
<td></td>
</tr>
<tr>
<td>PTC</td>
<td>9</td>
<td>6(66.7%)</td>
<td>3(33.3%)</td>
<td></td>
</tr>
<tr>
<td>PTCFV</td>
<td>10</td>
<td>6(60%)</td>
<td>4(40%)</td>
<td></td>
</tr>
<tr>
<td>PDTC</td>
<td>7</td>
<td>1(14.3%)</td>
<td>6(85.7%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lymph node metastases</th>
<th>No. of cases</th>
<th>Membranous</th>
<th>Cytoplasmic or nuclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>25</td>
<td>15(60%)</td>
<td>10(40%)</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>2(15.4%)</td>
<td>11(84.6%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Capsular invasion</th>
<th>No. of cases</th>
<th>Membranous</th>
<th>Cytoplasmic or nuclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>13</td>
<td>10(76.9%)</td>
<td>3(23.1%)</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>7(28%)</td>
<td>18(72%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood vessel invasion</th>
<th>No. of cases</th>
<th>Membranous</th>
<th>Cytoplasmic or nuclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>17</td>
<td>13(76.5%)</td>
<td>4(23.5%)</td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>4(19%)</td>
<td>17(81%)</td>
</tr>
</tbody>
</table>

A statistically significant correlation (p<0.05) between β- catenin expression and type of thyroid tumor, LN metastases, capsular and blood vessel invasion was found:

β- catenin expression is membranous in all hyperplastic, in 84.6% of benign follicular adenoma and 63.2% of carcinoma cases with papillary features, while it was mainly cytoplasmic in FC and poorly differentiated anaplastic carcinomas (65.7% and 85.7% respectively).

Membranous expression is more common in cases (60%) with negative LN metastases, negative capsular invasion (76.9%) and negative blood vessel invasion (76.5%), while cytoplasmic and/or nuclear expression is related to cases with positive LN metastasis, positive capsular invasion and positive blood vessel invasion (84.6%,72% and 81% respectively).
**Nuclear morphometric results:** Table (4)

Table (4): The relation of nuclear morphometric measures to tumor type, lymph node metastases, capsular and blood vessel invasion

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. of cases</th>
<th>Morphometric measures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MNA</td>
<td>MNEF</td>
</tr>
<tr>
<td>NG</td>
<td>9</td>
<td>30.32</td>
</tr>
<tr>
<td>FA</td>
<td>13</td>
<td>31.39</td>
</tr>
<tr>
<td>FC</td>
<td>12</td>
<td>34.79</td>
</tr>
<tr>
<td>PTC</td>
<td>9</td>
<td>35.18</td>
</tr>
<tr>
<td>PTCFV</td>
<td>10</td>
<td>39.09</td>
</tr>
<tr>
<td>PDTCA</td>
<td>7</td>
<td>39.70</td>
</tr>
</tbody>
</table>

**Lymph node metastases**

<table>
<thead>
<tr>
<th>Lymph node metastases</th>
<th>No. of cases</th>
<th>MNA</th>
<th>MNEF</th>
<th>LD/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>25</td>
<td>34.76</td>
<td>0.76</td>
<td>1.34</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>38.17</td>
<td>0.71</td>
<td>1.44</td>
</tr>
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**Capsular invasion**

<table>
<thead>
<tr>
<th>Capsular invasion</th>
<th>No. of cases</th>
<th>MNA</th>
<th>MNEF</th>
<th>LD/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>13</td>
<td>34.52</td>
<td>0.75</td>
<td>1.36</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>36.26</td>
<td>0.74</td>
<td>1.37</td>
</tr>
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**Blood vessel invasion**

<table>
<thead>
<tr>
<th>Blood vessel invasion</th>
<th>No. of cases</th>
<th>MNA</th>
<th>MNEF</th>
<th>LD/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>17</td>
<td>35.24</td>
<td>0.75</td>
<td>1.35</td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>36.09</td>
<td>0.74</td>
<td>1.39</td>
</tr>
</tbody>
</table>

MNA= Mean nuclear area
MNEF= mean nuclear elongation factor (small diameter/ long diameter)
LD/SD= long diameter/ small diameter

A statistically significant correlation between MNA and type of thyroid tumor (p<0.05) was found. Benign lesions had a significantly lower MNA than carcinomas (Fig 6, 7, 8). Also, poorly differentiated carcinoma had higher LD/SD (Fig 9) than other types of thyroid carcinomas (p<0.05).

However, correlations between MNA and other variables (L.N. metastasis, capsular invasion and vascular invasion) were statistically insignificant (p>0.05).

Other calculated morphometric measures (MNEF & LD/SD) had statistically insignificant correlations with the clinicopathological variables examined (p>0.05).
**Fig (1):**- a case of papillary thyroid carcinoma, showing high score of cytoplasmic brown staining of malignant tumor cells for galectin-3, (streptavidin/biotin DAB, x400).

**Fig (2):**- a case of papillary thyroid carcinoma follicular variant, showing moderate cytoplasmic brown staining of malignant tumor cells for galectin-3, (streptavidin/biotin DAB, x400).
Fig (3):- a case of follicular thyroid carcinoma, showing high score of cytoplasmic brown staining of malignant tumor cells invading the capsule for galectin-3 (streptavidin/biotin DAB, x100).

Fig (4):- a case of papillary thyroid carcinoma follicular variant, showing high score of membranous brown staining of malignant tumor cells for B-catenin, (streptavidin/biotin DAB, x400).
Fig (5): a case of follicular thyroid carcinoma, showing high score of cytoplasmic brown staining of malignant tumor cells for B-catenin, (streptavidin/biotin DAB, x200).

Fig (6): a case of follicular thyroid carcinoma, showing: a) Min & Max diameters with MNEF: 0.74 b) MNA: 34.79 c) LD/SD: 1.41 (H&E, x400 with 400% zooming).
**Fig (7):** A case of papillary thyroid carcinoma, showing: a) Min & Max diameters with MNEF: 0.74   b) MNA: 35.18   c) LD/SD: 1.34   (H&E, x400 with 400% zooming).

**Fig (8):** A case of papillary thyroid carcinoma follicular variant, showing: a) Min & Max diameters with MNEF: 0.78   b) MNA: 39.09   c) LD/SD: 1.28   (H&E, x400 with 400% zooming).
Fig (9):- a case of poorly differentiated thyroid carcinoma, showing: a) Min & Max diameters with MNEF: 0.56  b) MNA: 39.70  c) LD/SD: 1.8 (H&E, x400 with 400% zooming)

Discussion:

One of the critical steps in cancer metastasis is the adhesion of disseminating tumour cells to the blood vessel endothelium in distant organs. This process is thought to be regulated by the mechanical properties of the cancer cells and also by the specific expression of various adhesion molecules and/or ligands to adhesion molecules on the surface of cancer cells and endothelial cells (Miles, et al; 2008).

Galectin-3 is a beta-galactoside-binding protein that regulates many biologic processes, including cell adhesion, migration, cell growth, tumor progression, metastasis, and apoptosis (Bartolazzi, et al; 2008 and Chiu, et al; 2010). The expression of Gal-3 was studied immunohistochemically in thyroid lesions, with the aim of studying its reliability as a diagnostic indicator, particularly in differentiating problematic cases that are inconclusive with routine H&E staining technique. These include PTCFV and minimally-invasive FCA. By doing so, it is supposed to facilitate surgical management and treatment.

In this study, Gal-3 was found over-expressed in all malignant thyroid neoplasms. However, it was not or weakly detectable in normal and non-malignant tissue including follicular adenoma, supporting the results of Xu, et

In the present study, Gal-3 was expressed in nodules with cytological atypia, and could thus provide a valuable clue in the detection of lesions of undetermined malignant potential, like in nodules with an overall benign appearance but with focal areas suspicious for malignancy. It was also observed that Gal-3 may be valuable in differentiating a non-malignant hyperplastic papilla from that of a papilla formed in PTC in which a strong expression of galactin-3 was detected, suggestive to be useful in the early detection of occult PTC in hyperplastic goitre. This is supported by Gong, et al (2012) and Paunovic, et al (2012) who found that the expression of this individual marker was most helpful for the diagnosis of papillary carcinoma.

With a strong expression of Gal-3 in PTC, we believe that Gal-3 is a good indicator in early detection of malignant transformation. Cytoplasmic predominance expression of galectin-3 has been related to progression of normal tissue to adenoma and carcinoma in the colon carcinoma model (Lotan et al, 1991). Our data and previous studies suggest that the same pattern of cellular staining can be used with thyroid neoplasm.

Controversial work done by Jakubiak- Wielganowicz et al (2003) and Mehrotra et al (2004) showed that Gal-3 is not a reliable marker to distinguish benign from malignant thyroid lesions, and that it is not a highly specific marker in differentiating between follicular benign and malignant tumours. Also, Davies et al (2006) concluded that Gal-3 does not discriminate between FAs and carcinomas; it is neither specific nor sensitive enough to be used satisfactorily in clinical practice as a marker of thyroid malignancy. Furthermore, Papale, et al (2013) found that Gal-3 alone, as a molecular marker of thyroid cancer, can still have a limited application in pre-surgery diagnosis.

As observed by Coli et al. (2002), it was found in this work a high expression of Gal-3 in fibroblasts, endothelial cells, macrophages, histiocytes, red blood cells and inflammatory infiltrates. This observation could be applicable as an internal positive control.

A characteristic finding in the current study of high positive Gal-3 staining reaction, particularly in the capsular invading cells in FCA supports the study done by Kawachi et al (2000) and Cvejic et al., (2005) confirming
that this marker has a possible role in the invasive capacity of the tumor and metastasis formation.

Saggiorato et al (2001) revealed that Gal-3 is a reliable presurgical immunocytochemical diagnostic marker in minimally invasive FCA, improving accuracy of conventional fine-needle aspiration biopsy (FNAB). This is in accordance with the findings of the present study that Gal-3 is a valuable adjunct diagnostic indicator, preferably in PTC, and could be of great value in preoperative diagnostic FNAB.

In the current work, all cases with positive LN metastases, and with positive blood vessel invasion showed high expression of galectin-3 (score 2 and 3) which was statistically significant. Out of 25 cases with positive capsular invasion, 24 cases (96%) showed high expression for galectin-3, another statistically significant correlation. High Galactin-3 expression is related to presence of thyroid carcinomas, regardless the type of carcinoma.

This may be explained by study done by Zhao, et al (2009) showing that galectin-3, whose concentration is greatly increased in the circulation of cancer patients, increases cancer cell adhesion to macro- and micro-vascular endothelial cells under static and flow conditions, increases trans-endothelial invasion and decreases the latency of experimental metastasis in athymic mice. These effects of galectin-3 are shown to be a consequence of its interaction with cancer-associated MUC1, which breaks the “protective shield” of the cell surface MUC1 by causing MUC1 polarization leading to exposure of smaller cell surface adhesion molecules/ligands including CD44 and ligand(s) for E-Selectin. Thus, the interaction in the bloodstream of cancer patients between circulating galectin-3 and cancer cells expressing MUC1 bearing the galectin-3-ligand TF (Galβ1, 3GalNAc-) promote metastasis.

However, Gong, et al (2012) and Lee and Lee (2013) found that there was no difference in the expressions of galectin-3 in relation to lymph node status.

The present work supports previous findings that Gal-3 detection is a simple, cheap and an important diagnostic support in distinguishing malignant and benign thyroid neoplasms. The immunocytochemical method is preferred to other molecular biology-based methods which may show false-positive results Coli et al. (2002). Morphological evaluation associated with immunocytostaining with galectin-3 should be the best approach for presurgical evaluation for thyroid nodules.

βeta-Catenin is part of a membrane-bound cell growth-signaling complex that plays a role in cell adhesion, as well as in promotion of growth (Rezk et al., 2004).

Thyroid carcinomas in the current work are associated with decreased membrane expression of β-catenin. The reduction is significantly higher in carcinomas compared to follicular adenomas. Although the number of cases
analyzed is small, this observation may prove useful in the distinction between follicular adenoma and well-differentiated follicular carcinoma, a differential diagnosis that is extremely difficult on fine-needle aspiration specimens and can be problematic also on histology sections. β-catenin can be of a diagnostic utility for thyroid lesions, because it shifts from a membranous localization to a cytoplasmic localization in malignant lesions.

In the present study there was a dramatic down-regulation of membrane bound β-catenin in poorly differentiated anaplastic thyroid cancer that represents the least differentiated form of carcinoma and the end point of tumor progression. This study expands the preliminary observation of Garcia-Rostan, et al. (2001) and Rezk et al. (2004) who demonstrated that reduced membranous immunoreactivity closely parallels tumor formation and progressive loss of tumor differentiation. Reduced membrane β-catenin has been documented in numerous human cancers (Wijnhoven et al., 1999), where it was sometimes observed in high-grade or poorly differentiated tumors.

The close parallel between dysregulation of β-catenin and neoplastic progression is consistent with the concept of a continuum in the spectrum of thyroid tumor differentiation (Rezk et al., 2004).

In contrast with neoplasms of most other epithelial organs, thyroid carcinomas of follicular cell origin have traditionally been viewed in terms of extremes, ie, well-differentiated carcinomas with papillary or follicular morphology and undifferentiated carcinomas and this approach is still reflected in the current staging system for thyroid cancer (Fleming et al., 1997).

The correlations shown in this study between loss of tumor differentiation and β-catenin dysregulation suggest this marker may prove an objective and useful adjunct to the diagnosis of these thyroid tumors.

In the present study, membranous expression is more common in cases (60%) with negative LN metastases, negative capsular invasion (76.9%) and negative blood vessel invasion (76.5%), while cytoplasmic and/or nuclear expression is related to cases with positive LN metastasis, positive capsular invasion and positive blood vessel invasion. In a few studies (Ramesh et al., 1999) the correlation has been shown to be statistically significant. This reduction is consistent with progressive deregulation of intercellular adhesion in cancer that promotes tumor detachment from the primary site and facilitates tumor spread (Wijnhoven et al., 1999). In this series, reduced membrane β-catenin immunoreactivity parallels high tumor stage and an increased propensity of the cancer to spread locally outside the thyroid, to invade blood vessels, or give rise to distant metastases. These findings validate experimental models showing an inverse correlation between β-catenin levels and hematogenous spread of murine carcinoma cells (Akimoto et al., 1999) and are similar to reports in other tumor types where reduced β-catenin expression was also associated with the capacity to invade (Lou et al., 1999) and metastasize (Fujimoto et al., 1997). This must be related to the location and mechanism of
action of β-catenin. This study identifies β-catenin dysregulation as a pathway in the progression of thyroid tumors. Altered β-catenin is a marker for aggressive tumor phenotypes among neoplasms of thyroid follicular cell derivation because both reduction of β-catenin membrane immunoreactivity as well as its aberrant cytoplasmic and/or nuclear localization closely parallel loss of tumor differentiation and poor prognosis. The analysis of β-catenin expression or mutation status may ultimately be very useful to objectively subtype thyroid neoplasms and predict outcomes.

Computerized morphometry can enhance the interpretation of morphological features by the transformation of pathological changes in cells to a qualitative form (Sheng-Lan, et al, 2005). Nuclear morphometric features of malignant cells differ from that observed in non-malignant cell nuclei. This observation has led to the hypothesis that such changes occur prior to the emergence of clinically detectable disease and that nuclear morphometry can be used as a biomarker for estimating an individual’s risk for cancer (Boone, et al, 2000).

In this study, there was a statistically significant correlation between MNA and type of thyroid tumor (p˂0.05). Benign lesions had a significantly lower MNA than carcinomas. However, correlations between MNA and other variables (L.N. metastasis, capsular invasion and vascular invasion) were statistically insignificant, (p˃0.05).

Bakr, et al (2000) reported that the mean nuclear area (MNA) and mean nuclear elongation factor (MNEF) are statistically significant discriminators between thyroid carcinomas and adenomas and the combination of both parameters constitute an optimal discriminatory potential. Also, Wright, et al, (1987) detected significant differences in mean nuclear areas and nuclear perimeters between multinodular goitres and follicular and papillary neoplasms and significant differences between follicular adenomas and follicular and papillary carcinomas. Furthermore, Janković et al; (2011) concluded that preoperative morphometry is a useful method in differential diagnosis of thyroid carcinoma from benign lesions, and considered it as a complementary method to conventional cytodiagnostics.

On the other hand (Kefeli, et al, 2010) reported that there were no significant differences between the benign lesion and follicular neoplasia groups for any nuclear parameters. Also (Rajesh, et al, 2004) reported that there was overlap of morphometric parameters between follicular adenoma, follicular carcinoma and papillary carcinoma.

We believe that morphometric assessment alone is inadequate to predict malignancy in thyroid tumors.

References:

1. Akimoto T, Kawabe S, Grothry A, Milas L: Low E-cadherin and beta catenin expression correlates with increased spontaneous and artificial


8. **Casey MB, Lohse CM, Lloyd RV**: Distinction between papillary thyroid hyperplasia and papillary thyroid carcinoma by immunohistochemical staining for cytokeratin 19, galectin-3, and HBME-1. *Endocr Pathol.14*:55-60; 2003


24. **Giunti S, Antonelli A, Amorosi A and Santarpia L**: Cellular Signaling Pathway Alterations and Potential Targeted Therapies for Medullary


36. Lee YM and Lee JB: Prognostic value of epidermal growth factor receptor, p53 and galectin-3 expression in papillary thyroid carcinoma. *Journal of International Medical Research* published online 28 March 2013 in advance of the print journal.


45. **Mokhtar N, Adel I, Goda I**: Cancer Pathology Registry 2003-2004 and time trend analysis, Department of Pathology, NCI, 2007.


57. **Romitti M, Ceolin L, Siqueira DR, Ferreira CV, Wajner SM, Maia AL:** Signaling pathways in follicular cell-derived thyroid carcinomas. *International Journal of Oncology*. 42 (1); 2013


62. **Shih SR, MD, Chang YC, Li HY, Liau JY, Lee CY, Chen CM, Chang TC:** Preoperative prediction of papillary thyroid carcinoma prognosis with the assistance of computerized morphometry of cytology samples obtained by fine-needle aspiration:Preliminary report. *HEAD & NECK—DOI 10.1002/HED; JANUARY 2013*


تقييم كلا من جالاكتين-3 و بيتا كاتينين، وقياسات الشكل النووي في بعض إصابات الغدة الدرقية

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الهدف من البحث: يهدف هذا البحث إلى دراسة القياسات الخلوية (قياس الشكل التحليلي) في مختلف إصابات الغدة الدرقية، تقييم تركيز وكثافة وجود الجالاكتين-3 والبيتا- كاتينين في إصابات الغدة الدرقية المختلفة لاستخدامها في التشخيص وتحديد تطور الحالة (مستقبل الورم) الخاص بكل نوع من الأورام ومرحلةه.

الطريقة: شملت هذه الدراسة 60 عينة من أمراض الغدة الدرقية من بينهم 9 حالات سرطان الغدة الدرقية الحليمي، 10 السرطان الحليمي من النوع الغدي، 12 السرطان الغدي، 7 سرطان الغدة الدرقية فاقد التميز، 13 ورم غدي حميد و9 تضخم الغدة الدرقية العقدي. تم فحص نسيج الغدة الدرقية الطبيعي المجاورة للورم في 10 حالات. تم صبغ الحالات بالصبغة المناعية الهستوكماوية باستخدام مضادات دلالات الجالاكتين-3 والبيتا- كاتينين. أيضا تم فحص الشرائح المصبغة بالهيماتوكسيلين والأيوسين باستخدام برنامج محلل الصورة "بروبيلس ف". في قياس الصفات المورفولوجية وشملت متوسط قطر النويات، متوسط المحيط النووي ومتوسط المساحة النووية.
النتائج:

1- متوسط قطر الورم النووي و متوسط المحيط النووي كانت أعلى في حالات سرطان الغدة الدرقية فاقد التميز بالمقارنة مع الأنواع الأخرى وكانت الأقل حالات سرطان الغدة.

2- التعبير بدلاً من الورم النووي كاتيني منخفض في 15.4% من الأورام الغدية الحميدة وفي 60.5% من حالات سرطان الغدة الدرقية، وارتباط انخفاض التعبير بدلاً للبيتا- كاتيني مع فقدان التدريجي لتمييز الورم وزيادة الانتشار.

3- كان التفاعل المناعي للجالاكتين-2 أقوى في سرطان الغدة الدرقية الحميدة، في حين كان أقل كثافة في السرطان الحميدة من النوع الغدي، السرطان الغدي و سرطان الغدة الدرقية فاقد التميز، كما كانت التفاعلية المناعية ضعيفة ومتفرقة في حالات الورم الغدي الحميد وأضعف حتى في تضخم الغدة الدرقية العقد.

الاستنتاجات:

وخلصت الدراسة إلى أن التقدير الكمي للخصائص النووية يلعب دوراً في تقييم الملامح المورفولوجية ومن ثم في المساعدة في تشخيص أمراض الغدة الدرقية. كما وجُد أن دراسة كثافة البيتا- كاتيني والجالاكتين-2 مفيدة كمساعد للتمييز بين الأورام الغدة الدرقية الحميدة والسرطانية والتنبؤ بالنتائج وترتبط بشكل كبير بتدخُل الحالات.