The expression of CK19, vimentin and E-cadherin in differentiated thyroid carcinomas

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Abstract
Thyroid carcinomas constitute lesions with an ascending incidence, for which many prognosis assessment systems were developed. This study focused on analyzing the immunoexpression of CK19, vimentin and E-cadherin in a number of 43 differentiated thyroid carcinomas, of which 39 papillary carcinomas and four follicular carcinomas, and assessed the relationship of these markers with clinico-pathological parameters of interest, such as age and gender of patients, the histological type and subtype, tumor size and extension, metastases in regional lymph nodes and tumor stage. CK19 immunostaining indicated higher scores in conventional and follicular papillary carcinomas compared with tall cell variant. In relation to the size and extension of the tumor, we found significantly higher values of vimentin and E-cadherin scores in T1–T2 carcinomas compared with T3–T4 category and a positive linear distribution of these markers, which sustain their involvement in common mechanisms of tumor progression.

Keywords: differentiated thyroid carcinomas, CK19, vimentin, E-cadherin.

Introduction
Thyroid cancer although rare, is the most common type of endocrine malignancy, accounting for approximately 1.5% of all newly diagnosed cancers in the United States, its incidence being steadily increasing worldwide in the last three decades [1].

Differentiated thyroid cancers, papillary and follicular usually have a favorable prognosis on the long term despite frequent involvement of lymph nodes in papillary cancer [2], or the frequency of distant metastases in follicular cancer. However, some patients have an increased risk of relapse and even death. These patients could be identified at the time of diagnosis by using the prognostic factors, including the characteristics of the patient (age, gender), tumors (histology, sizes, aneuploidy) and treatment [3].

Similar to other malignancies, the genesis and development of thyroid carcinomas is correlated with various oncogenes and tumor suppressor genes such as ras gene [4], ret gene [5], p53 [6] and also metastasis associated genes such as NM23 [7], CD44v6 [8] and EGFR [9]. Although there have been described several types of oncogene changes as a possible mechanism in thyroid tumorigenesis, it is still unclear whether these changes can serve as markers for assessing the biological behavior of thyroid tumors or to confirm their diagnosis.

Until now, in many cases, traditional histopathology was not appreciated to assess the potential aggressiveness of these carcinomas. As a result, several studies based on immunohistochemical techniques aimed to identify markers that allow selection of cases with unfavorable evolution.

We followed the immunohistochemical expression of CK19, vimentin and E-cadherin in differentiated thyroid carcinomas and assessed the possible relation of their expression with clinico-pathological factors as age and gender of patients, histological type and subtype, tumor sizes and extension, metastases in lymph regional nodes and tumor stage.

Materials and Methods
Our study included a total of 43 differentiated thyroid carcinomas, of which 39 papillary carcinomas and four follicular carcinomas, from patients operated in Surgery Clinics of Emergency County Hospital, Craiova, Romania, between the years 2003–2011.

The biological material consisted of surgical excision samples, fixed in 10% buffered formalin, processed by the usual histological paraffin embedding technique and Hematoxylin–Eosin (HE) staining. The classification of lesions was performed in accordance with the criteria elaborated by the AJCC (American Joint Committee on Cancer) in 2010 [10].

Immunohistochemical analysis was performed on serial sections and the antibodies panel that has been used is shown in the table below (Table 1).

For the immunohistochemical analysis, we used LSAB2–HRP amplification system (code K0675, Dako), using 3,3’-Diaminobenzidine (DAB) as chromogen for visualization (code 3467, Dako). To validate the reactions, we used positive external controls (data not shown) and negative external controls by omitting the primary antibody.
Table 1 – Panel of antibodies used for the immunohistochemistry

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone/ Source</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>External positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19</td>
<td>RCK108/ Dako</td>
<td>1/150</td>
<td>HIER, Citrate</td>
<td>Spleen</td>
</tr>
<tr>
<td>Vimentin</td>
<td>SP20/Thermo Scientific</td>
<td>1/50</td>
<td>PIER, Pepsin</td>
<td>Prostate</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>NCH-38/ Dako</td>
<td>1/100</td>
<td>HIER, Citrate, Mammary epithelium</td>
<td></td>
</tr>
</tbody>
</table>

HIER: Heat Induced Epitope Retrieval; PIER: Proteolytic Induced Epitope Retrieval.

For the semiquantitative quantification of the analyzed markers, we used an adapted scoring system based on the literature data [11–13], which assessed the intensity of the reactions and the percentage of the labeled cells. Thus, the reactions were considered to be intense (score 3), respectively moderate (score 2) and weak (score 1); the threshold of reaction positivity was 5%, and we set four groups in order to assess the percentage of labeled cells (score 1 – 5–25%, score 2 – 26–50%, score 3 – 51–75%, score 4 – more than 75%). Composite score was obtained by multiplying the intensity scores with the percentage of labeled cells, obtained values ranging from 1 to 12. For the statistical analysis, the composite score was considered low for 1–4 score, medium for 5–8 score, and high if the score was 8–12.

For the statistical analysis, we used $\chi^2$ test, One-Way ANOVA test, Fisher’s exact test and Pearson’s correlation index using the SPSS 10 software and values of $p<0.05$ were considered significant. Image acquisition was performed using a Nikon Eclipse E600 microscope and Lucia 5 software.

Results

We analyzed a total of 43 differentiated thyroid carcinomas diagnosed in patients predominantly females (M/F=4/39), aged between 22–82 years, with an upward trend since the third decade of life. Clinico-pathological characteristics are presented in the Table 2.

Table 2 – CK19, vimentin and E-cadherin immunoexpression statistical analysis according to the investigated clinico-pathological parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>CK19</th>
<th>Vimentin</th>
<th>E-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average values</td>
<td>p*</td>
<td>Average score</td>
<td>p*</td>
</tr>
<tr>
<td>Age [years]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45 = 16</td>
<td>9.0</td>
<td>0.179</td>
<td>2.6</td>
</tr>
<tr>
<td>≥45 = 27</td>
<td>8.6</td>
<td>2.5</td>
<td>8.2</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females = 39</td>
<td>8.8</td>
<td>0.884</td>
<td>2.7</td>
</tr>
<tr>
<td>Males = 4</td>
<td>8.7</td>
<td>3.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Tumor types</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary = 39</td>
<td>9.0</td>
<td>0.000</td>
<td>2.7</td>
</tr>
<tr>
<td>Follicular = 4</td>
<td>2.0</td>
<td>2.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Histological variants (papillary carcinoma)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional = 23</td>
<td>8.9</td>
<td>0.000</td>
<td>3.0</td>
</tr>
<tr>
<td>Follicular = 13</td>
<td>9.2</td>
<td>2.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Tall cell = 3</td>
<td>0.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>T category (T1–T2/T3–T4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 = 7</td>
<td>8.5</td>
<td>4.0</td>
<td>10.3</td>
</tr>
<tr>
<td>T2 = 27</td>
<td>8.7</td>
<td>3.0</td>
<td>9.1</td>
</tr>
<tr>
<td>T3 = 4</td>
<td>9.0</td>
<td>1.7</td>
<td>4.3</td>
</tr>
<tr>
<td>T4a = 5</td>
<td>9.6</td>
<td>1.5</td>
<td>4.2</td>
</tr>
<tr>
<td>N category (lymph node metastasis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0 = 33</td>
<td>8.6</td>
<td>0.548</td>
<td>2.5</td>
</tr>
<tr>
<td>N1 = 10</td>
<td>9.2</td>
<td>2.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Tumor stage (I–II/III–IV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I = 15</td>
<td>8.5</td>
<td>2.7</td>
<td>9.0</td>
</tr>
<tr>
<td>II = 15</td>
<td>9.3</td>
<td>2.6</td>
<td>8.2</td>
</tr>
<tr>
<td>III = 7</td>
<td>8.3</td>
<td>1.7</td>
<td>8.6</td>
</tr>
<tr>
<td>IV = 3</td>
<td>10.0</td>
<td>1.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Primitive tumor vs. metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primitive = 10</td>
<td>9.2</td>
<td>0.865</td>
<td>2.8</td>
</tr>
<tr>
<td>Metastasis = 10</td>
<td>9.6</td>
<td>2.7</td>
<td>0.763**</td>
</tr>
</tbody>
</table>

$p^*$: $\chi^2$ correlation level; **: Fisher’s exact test.

Histologically, the 43 differentiated thyroid carcinomas were papillary carcinoma in the 39 cases (23 cases conventional form, follicular variant 13 cases, tall cell variant three cases) and follicular carcinoma in four cases (three cases conventional form, cell clear variant one case). The sizes of the tumors ranged between 0.8–12 cm, invasion of adjacent structures (tracheal wall) being present in five cases of papillary carcinomas of which three cases of tall cell variant and two cases of follicular variant. Metastases in regional lymph nodes (pre- or paratracheal) we observed in10 cases of papillary carcinomas of which five cases of conventional variant, three cases of follicular variant and tall cell variant in two cases. Most of the investigated thyroid carcinomas were classified in stage I and II of disease, with 17 and respectively 16 cases, while seven cases corresponded to stage III and three cases to stage IVa.

CK19 immunoreaction

CK19 immunoreaction was identified in the cytoplasm, increased in the perinuclear area in 84.6% of papillary carcinomas (Figure 1A) and with a diffuse cytoplasmic pattern in 25% of the follicular carcinomas (Figure 1B). In the case of papillary carcinomas, the reaction was intense with an average percentage of labeled cells of 79.8% and an average score of 9.0, compared with the positive follicular carcinomas, which presented a moderate intensity, 10% of labeled cells and a score of 2.0, differences
which were statistically significant (p<0.001, \( \chi^2 \) test). Also, in relation to the variants of papillary carcinoma, we found significant differences in the expression of CK19 in conventional and follicular carcinomas, compared with the tall cell variant (p<0.001, \( \chi^2 \) test). Thus, in the case of conventional and follicular variant, the immunostaining was intense, with mean percentage values of 80.3% and 78.6%, and the average scores of 8.9, respectively 9.2, while for the tall cell variant the reaction was negative in all cases. We found no differences of CK19 immunostaining, depending on conventional and follicular variants of papillary carcinoma.

Statistical analysis indicated no significant difference for CK19 expression in relation to age, gender, tumor size and extent, presence of lymph node metastasis and tumor stage (p>0.05, \( \chi^2 \) test).

**Vimentin immunostaining**

Vimentin immunostaining was identified being basal subnuclear in 53.8% of papillary carcinomas (Figure 2A) and diffuse cytoplasmic in 75% of the follicular carcinomas (Figure 2B), without significant differences between the two types of lesions (p>0.05, \( \chi^2 \) test).

For the vimentin-positive cases, One-Way ANOVA test indicated significant differences of labeled cells percentage (p<0.001) in conventional and follicular variants of papillary carcinoma, compared with tall cell variant (Figure 2C). Thus, in case of conventional and follicular variants, the reactions indicated moderate intensity, mean percentage values of 43.3% and 39.8%, and average scores of 3.0, respectively 2.8, compared with tall cell variant, in which the vimentin immunoreaction had a weak intensity, with an average of 12.3% labeled cells and an average score of 1.0.

The analysis of vimentin immunostaining indicated significant differences depending on the size and extent of tumor (T category) (p=0.039, \( \chi^2 \) test). Thus, in case of T1–T2 tumors, the mean percentage of labeled cells were 47.3% respectively 44.9%, and the average scores 4.0, respectively 3.0. Compared to these, for the categories T3–T4, the values were 30.7% and 25.7%, respectively 1.7 and 1.5.

We found no significant differences of vimentin immunostaining with age, gender, presence of lymph node metastasis and tumor stage (p>0.05, \( \chi^2 \) test).

**E-cadherin immunoexpression**

E-cadherin immunoexpression has been identified at the membrane and cytoplasm level in 84.6% of papillary carcinomas (Figure 2D), and with a membrane-only localization in 75% of analyzed follicular carcinoma (Figure 2E) without significant differences of the immunostaining (p>0.05, \( \chi^2 \) test). The analysis of the obtained reactions in relation to variants of papillary thyroid carcinoma indicated significant differences (p<0.001, \( \chi^2 \) test), the highest scores being present in conventional and follicular carcinomas, respectively 9.6 and 8.4 compared to tall cell variant (Figure 2F) where the average score was 1.0. The intensity of the reactions in case of conventional and follicular variants was increased, and the average percentages of labeled cells were 76.5% and 73.6%, significantly higher compared with tall cell variant, where the reactions were weak, with an average percentage of labeled cells of 14.3% aspects indicated also by One-Way ANOVA test (p<0.001).

Furthermore, we found significant differences of E-cadherin immunostaining depending on tumor size and extension (category T) (p=0.002, \( \chi^2 \) test). Thus, for the T1–T2 tumors, the mean percentage of labeled cells was of 76.1% and respectively 77.7%, and the average scores of 10.3, respectively 9.1. Compared to these, for T3–T4 categories the correspondent values were of 43.3% and 38.2%, respectively 4.3 and 4.2.

In this study, we found no significant differences of E-cadherin reaction in relation to age, gender, presence of lymph node metastasis and tumor stage (p>0.05, \( \chi^2 \) test). Also, we found no differences in CK19, vimentin and E-cadherin immunostainings in relation to the histological subtypes of follicular carcinomas.

The analysis of the percentage values of vimentin and E-cadherin positive cases using Pearson’s test indicated a positive linear distribution and a significant correlation for the two markers, indicating a similar variation for the studied group [r(41)=0.318, p=0.038] (Figure 3).

In this study, we performed a comparative analysis of CK19, vimentin and E-cadherin expression in metastases compared with the primitive tumors. The CK19 immuno-
staining in the thyroid lymph node metastasis was intense, with a mean of 78.1% labeled cells and the average score of 9.6, whereas the vimentin reaction was weak, with an average of labeled cells of 38.2% and an average score of 2.7. E-cadherin immunostaining was negative in all examined lymph node metastases, representing the only statistically significant relation compared to the corresponding primitive tumors ($p=0.001$, $\chi^2$ test).

**Discussion**

Cytokeratin 19 (CK19) is a high molecular weight cytokeratin, a sensitive (but non-specific) marker of papillary thyroid carcinoma [14]. Therefore, the absence of immunostaining for CK19 is an argument against this diagnosis.

CK19 expression analysis indicated positivity in 59.9% of the analyzed thyroid carcinomas, with a percentage of 94.8% for papillary carcinomas and only of 25% for follicular carcinomas. Similar studies have reported closely-related results for CK19 positivity, respectively in 96% of papillary carcinomas and 14% of follicular carcinoma [14], 100% of papillary carcinomas and 68% of follicular carcinomas [15], 72.3% of the papillary carcinomas and of follicular carcinomas 4% [16].

![Image](image_url)

**Figure 2** – Vimentin immunostaining, ×100: (A) Papillary thyroid carcinoma, conventional variant; (B) Follicular thyroid carcinoma, conventional variant; (C) Papillary thyroid carcinoma, tall cell variant. E-cadherin immunostaining, ×100: (D) Papillary thyroid carcinoma, conventional variant; (E) Follicular thyroid carcinoma, conventional variant; (F) Papillary thyroid carcinoma, tall cell variant.
Regarding the expression pattern of CK19, we found that in papillary carcinomas, except tall cell variant, and in their lymph node metastases, the staining was intense cytoplasmic in the perinuclear region in more than 75% of tumor cells. Unlike this, in follicular carcinomas the immunostaining was diffuse cytoplasmic, but with moderate intensity and present in 10% of tumor cells. There were significant differences in the expression of CK19 in conventional and follicular carcinomas compared with tall cell variant \( (p<0.001, \chi^2 \text{ test}) \), and with no significant expression differences for this marker in relation to age, gender, size and extent of tumor nodes metastases and tumor stage \( (p>0.05, \chi^2 \text{ test}) \). Several studies have emphasized the importance of CK19 staining distribution and intensity, as the most important aspects for a more accurate diagnostic interpretation \[12, 16, 17\]. Immunoreactivity for CK19 is present in both types of differentiated thyroid carcinomas, but the scale and intensity of staining is higher in follicular carcinomas \[12\]. Miettinen et al. observed diffuse CK19 reactivity in all papillary carcinomas and about 50% of follicular carcinomas \[18\]. Kragsterman et al. concluded that CK19 has limited value as a diagnostic marker in thyroid tumors, but acknowledged that its positivity should raise suspicion of papillary thyroid carcinoma \[19\]. Moreover, strong and diffuse staining for CK19 in a thyroid tumor with follicular growth pattern should raise suspicion of follicular variant of papillary carcinoma, and the lesion should be examined carefully to identify the presence of nuclear characteristics \[12\].

In the study, we found vimentin positivity in 53.8% of papillary carcinomas and in 75% of follicular variant, with no significant differences between the two lesions.

It was found that overexpression of vimentin in cancers correlates with tumor increase and invasiveness and also with metastasis in various locations, the protein being present in the tumor epithelial cells during the epithelial-mesenchymal transition process \[20\].

Vimentin expression pattern analysis indicated basal subnuclear positivity in papillary carcinomas and diffuse cytoplasmic in follicular carcinomas. Compared with conventional variants, tall cell variant revealed a membrane immunolocalization of vimentin, with weak intensity. We observed significant differences in the expression of vimentin in conventional and follicular variants of papillary carcinoma, compared with tall cell variant of papillary carcinomas \( (p=0.001) \). We found no significant differences for vimentin staging with age, gender, presence of lymph node metastasis and tumor stage \( (p>0.05, \chi^2 \text{ test}) \).

Yamamoto et al. found in thyroid papillary carcinomas a weak and focal expression of vimentin in the case of lesions with distant metastasis compared with those without metastases \[21\]. On the other hand, in some carcinomas as in the case of prostatic location, vimentin is overexpressed, at least in some of the cell lines, which is supposed to be a marker for invasiveness and metastasis and is involved in the increase of cell motility \[22, 23\]. Loss or reduction of cell adhesion by abnormal expression level or function of E-cadherin were often described in human cancers \[24, 25\]. Regulation of the expression of E-cadherin gene has been linked to the promoter methylation status in multiple tumor models, including prostate cancer \[26\], breast cancer \[26\], and thyroid cancer \[27\].

In this study, the positivity for E-cadherin was present in 87.3% of the cases analyzed, of which 84.6% in papillary carcinomas and in 75% of follicular carcinomas. Similar studies communicate similar percentages of positivity in differentiated thyroid carcinoma: 91% \[28\], 90% \( (80.3\% \text{ papillary carcinomas, } 100\% \text{ follicular carcinomas}) \[29\], 89.6% \( (79.2\% \text{ papillary carcinomas, } 100\% \text{ follicular carcinomas}) \[30\], 85.4% \( (87.5\% \text{ of papillary carcinomas, } 83.3\% \text{ follicular carcinomas}) \[31\], 83.9% \[32\], 80% \[33\].

E-cadherin expression pattern in conventional and follicular variants of papillary carcinoma was membranous (sometimes cytoplasmic and membranous) with high intensity, being membranous and with low intensity for the tall cell variant, and absent in lymph node metastases of papillary carcinoma. For follicular carcinomas, the expression was similar to that of conventional papillary carcinomas. We have also found significant differences in staining of E-cadherin related to the size and extent of tumor \( (p=0.002, \chi^2 \text{ test}) \). It is known that a reduced expression of E-cadherin correlates with progression, aggressivity and reduced survival of several types of carcinomas \[34, 35\]. In this study, a complete loss of E-cadherin expression was correlated with tumor metastatic activity. Brabant et al. reported similar issues \[31\]. Several studies evaluating the expression of E-cadherin in thyroid carcinomas have focused on the relation of its expression with tumor size, lymph node involvement or metastases, but the reported results are not consistent \[28, 29, 31–33, 36–38\]. Some of these reports assessed the expression of E-cadherin in relation to histological subtypes of papillary thyroid cancer or with local invasion model (minimally invasive versus widely invasive) in follicular thyroid cancers \[39–43\].

Yin et al. (2007) reported that E-cadherin positivity rate is significantly lower in tumors with lymph node metastasis group than in the tumors group without lymph node metastasis \[44\]. Loss of E-cadherin expression and epithelial–mesenchymal transition may be transient, reversible, possibly regulated by the tumor microenvironment, and neoplastic cells that have undergone epithelial–mesenchymal transition during invasion seem to regain E-cadherin expression and epithelial characteristics of cohesion in secondary tumors \[45\]. During the colonization of distant sites, a reverse transition process occurs, of epithelial–mesenchymal transition and metastatic cancer cells regain epithelial phenotype \[46\].
In our study, there was a linear relation between the expressions of vimentin and E-cadherin, the molecules showing a weaker expression in larger and invasive carcinomas. Some studies indicated that vimentin regulates expression of intercellular adhesion molecules, respectively E-cadherin/β-catenin complex through C-src pathway [47]. Although the role of vimentin is not fully known, the existence of these regulating biomolecular pathways is fully recognized, and vimentin is currently considered as a potential therapeutic target [20].

Conclusions

The correlation of E-cadherin and vimentin expressions in primitive and metastatic differentiated thyroid carcinomas supports the existence of common mechanisms involved in tumor progression. Since the investigated molecules are potential targets for pharmacological agents, these findings open new therapeutic possibilities in differentiated thyroid carcinomas.

References


The expression of CK19, vimentin and E-cadherin in differentiated thyroid carcinomas


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