ASSESSMENT OF PLASMA B-CATENIN CONCENTRATION AS BIOMARKER OF THYROID CANCER*

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New diagnostic methods for thyroid diseases are still being searched for. Immunohistochemical diagnosis is expanded by the introduction of new biomarkers including β-catenin (B-Cat). Associations are indicated between the cellular expression of this biomarker and tumor stage, nodal metastases and the degree of tumor cell differentiation. Reports are scarce regarding the plasma level of this biomarker in malignant neoplastic diseases.

The aim of the study was to analyze the plasma B-Cat concentration and the possibility of its use in the diagnostics of patients with nodular goiter and papillary thyroid carcinoma.

Material and methods. Plasma B-Cat concentration was determined in 64 patients with goiter and 15 healthy volunteers. The final histopathological examination revealed 41 cases of papillary thyroid carcinoma (PTC) and 13 cases of nodular goiter (NG).

Results. A significant increase in B-Cat ($p < 0.05$) in both groups compared to the control group. No differences in the concentrations of biomarker was demonstrated between the PTC and NG groups. After determining the AUC for the tested biomarker, the B-Cat ratio of the area value 0.721 was the strong diagnostic test.

Conclusions. Changes in the plasma B-Cat concentration can be the biomarker of thyroid cancer but it cannot be used for the detection of papillary thyroid carcinoma because of concomitant tumor-like lesions in the thyroid gland.

Key words: beta-catenin, thyroid cancer

The extent of thyroid cancer surgery is radical and includes a total thyroidectomy and lymph node lymphadenectomy. A fine needle aspiration biopsy (FNAB) is the basic diagnostic test in preoperative detection of thyroid cancer. Differentiation of thyroid cancer histopathological specimens based on immunohistochemical staining confirms the possibility of β-catenin (B-Cat) application.

It results from the structure of B-Cat molecule that, depending on the structure of the molecule, it demonstrates the activity of signaling or adhesive protein (1). B-Cat binds adhesion proteins – cadherins and participates in intercellular signaling. In the nucleus, a cadherin-independent pool of B-Cat transduces Wnt signals by interacting with T-cell transcription factor (TCF). By dependencies β-catenin/cadherin and β-catenin/TCF it promotes tumorigenesis (2). Evoking smaller epithelial cell adhesion to the surface and their interaction after B-Cat combination with TGF-β play an important role in the development of metastases (3). Mutations in CTNNB1 gene (the gene encoding B-Cat) have been detected in several tumors (4).

The ability to detect thyroid cancer on the basis of the concentration of the above mentioned plasma biomarker would allow the classification of patients for radical surgery with the use of less invasive methods.

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The aim of the study was to determine the concentrations of B-Cat in the plasma of patients with thyroid cancer (nontoxic goiter, papillary thyroid carcinoma) and to determine the diagnostic usefulness of tests measuring their concentration in the preoperative diagnostics.

MATERIAL AND METHODS

Patients diagnosed with thyroid cancer or with a strong suspicion of malignancy based on the outcome of fine needle aspiration biopsy (FNAB) were enrolled into the prospective study. The patients who underwent thyroidectomy and central compartment neck dissection, in accordance with the principles of radical surgery of thyroid cancer were qualified for primary thyroid surgery at the Department of Endocrine and General Surgery, Medical University of Łódź. None of the patients underwent chemotherapy, radiotherapy or surgery of the thyroid prior to the enrollment. Table 1 presents a detailed characteristics of the study and the control groups. Mean age of the patients was 49.2±0 years; 67.7% of the examined population were women. The control group (CG) comprised 15 healthy volunteers, mean age 48.8±4.4 years; 73.3% of the examined population were women, in whom thyroid disease was excluded on the basis of medical history and physical examination. Forty one patients with papillary carcinoma (PTC) and 13 with nodular goiter (NG), in whom final histopathological examination excluded malignant lesions and who were diagnosed with benign thyroid adenoma, were included in the study. Groups were homologous as regards age and sex distribution (p <0.05). Due to the low number of respondents, ultimately the results obtained from the blood of patients with follicular, anaplastic and medullary cancer were rejected.

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Fasting blood was collected on the day of surgery from the cubital vein between 7:00 and 8:00 am. Within 30 minutes after collection, blood samples were centrifuged for 6 minutes at 6000 rpm. The collected plasma was frozen at -80 ° C. The determinations of plasma B-Cat concentrations were performed in ‘STI Laboratory in Poznan with the use of ELISA (enzyme linked immunoasay) commercial kit: Human Catenin Beta-1 (CTNNB1/CTNNB/OK/SW-cl.35/PRO2286) ELISA Kit Catalog Number. CSB-E08963h, kit of sensitivity <3.9 pg/mL, within-run precision (CV) 8% and between-run precision (CV) 10%, measuring concentrations in the range from 15.6 to 1000 pg/mL. All the determinations were performed once. For protein the used plasma samples were diluted respectively 5x for β-catenin (acc. to the producer’s guidelines), plasma protein concentrations calculated in the study include the applied dilutions.

The analyses were performed with STATISTICA 10 (academic license). Mean values were calculated and mean standard deviation (SEM). The differences between the mean values were examined, the statistical significance between the two groups of patients was determined using the Student’s t-test. The differences were considered statistically significant when p <0.05. The Pearson correlation coefficient – r was determined to investigate the correlations between variables. The regression equation defining the association between the variables was determined. Ability of the investigated parameter (B-Cat) to be used as a diagnostic test was determined using AUC (area under curve) – to be used as a diagnostic test. The area under the AUC above 0.6 was assumed to be reliable.

RESULTS

Table 2 presents results of plasma concentration ± SEM for B-Cat in the PTC and the NG group compared to the controls.

The analysis of mean plasma B-Cat concentrations demonstrated in the PTC and NG groups a statistically significant increase of the concentration of this biomarker as compared to the mean values in the control group. No statistically significant differences was found in mean B-Cat concentrations between PTC and NG groups.

The ability of the investigated parameter (B-Cat) to be used as a diagnostic test for the detection of thyroid cancers was determined using AUC. Calculated test obtained reliable values of the AUC > 0.6. AUC values for the test taking into account the use of the investi-
gated biomarker was the following 0.721 for B-Cat (fig. 1).

DISCUSSION

Nodular goiter and thyroid cancer are one of the most important problems of endocrine surgery. According to the data of the National Cancer Registry administered by the Centre of Oncology in Warsaw, papillary thyroid carcinoma is the most common histopathological type of thyroid cancer. In recent years, Poland has recorded an increase in new cases of thyroid cancer from 2071 cases in the year 2009 to 2641 cases three years later (5). Due to the differences in the completeness of surgical treatment of benign and malignant thyroid tumors, the effects and complications (postoperative bleeding, respiratory failure, vocal folds paralysis, laryngeal edema, unilateral or bilateral laryngeal nerve paralysis) appear to be of importance (6). Nodular goiter surgery does not require complete resection of the thyroid gland, which is performed when thyroid cancer is suspected. In practice it should be taken into account in any thyroid. There may come to the detection of thyroid cancer in the histopathological material after the surgery. In the case of incomplete thyroidectomy reoperation may be necessary and it is associated with an increased risk of postoperative complications described above.

The number of routinely determined thyroid cancer markers is limited to a few. Thyroglobulin is used in postoperative follow-up of differentiated thyroid cancer, whereas calcitonin is estimated in medullary carcinoma (7). Still, there are no biomarkers the detection of which would contribute to preoperative diagnostics of thyroid. Thus, the search for the methods of more and more sensitive and accurate diagnostics of neoplastic lesions in thyroid parenchyma as regards their malignancy is understandable. Reports concerning immunohistochemical evaluation of postoperative material in the case of suspected thyroid cancer suggest the determination of FN-1 or B-Cat in the. These studies define a new path that the modern diagnostics should follow (8-14).

The role of β-catenin in a cell has been described in association with many malignancies. First of all, there has been reported the relationship of this biomarker with Wnt pathway

<table>
<thead>
<tr>
<th>Investigated groups</th>
<th>Size</th>
<th>Gender</th>
<th>Age±SEM (years)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>females (%)</td>
<td>males (%)</td>
</tr>
<tr>
<td>CG</td>
<td>15</td>
<td>73,3</td>
<td>26,7</td>
</tr>
<tr>
<td>NG</td>
<td>13</td>
<td>69,2</td>
<td>30,8</td>
</tr>
<tr>
<td>PTC</td>
<td>41</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>FTC</td>
<td>4</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>ATC</td>
<td>3</td>
<td>33,3</td>
<td>66,7</td>
</tr>
<tr>
<td>MTC</td>
<td>3</td>
<td>66,7</td>
<td>33,3</td>
</tr>
<tr>
<td>Patients total</td>
<td>64</td>
<td>67,7</td>
<td>32,3</td>
</tr>
</tbody>
</table>

Fig. 1. AUC (area under) for the biomarker of B-Cat – assesses the chances for detection of thyroid cancer on the basis of the patient’s plasma sample test.
Assessment of plasma β-catenin concentration as biomarker of thyroid cancer and its impact on the development of malignant and benign tumors (15). A relationship has been indicated between B-Cat expression and the cancer stage in patients with colorectal, lung, urinary tract carcinomas or glioma (16, 17, 18).

An interesting report concerns one of the studies carried out in a Polish center. It has been shown that high level of nuclear B-Cat before the treatment is an unfavorable independent prognostic factor in patients with colorectal cancer. Median survival time in this group was twice lower with elevated level of nuclear B-Cat than in patients with normal concentration (12).

Different results were obtained in a study from Łódź, which determined B-Cat in prostate cancer cells. Nowicki (2010) showed a relationship between the degree of prostate cancer cell differentiation (Gleason scale), patients’ survival and the level of B-Cat expression in immunohistochemical examination. The level of B-Cat in prostate cancer cells was lower than in normal cells (16, 19). Similar results were obtained in reports from France of using B-Cat in the diagnostics of patients with bladder carcinomas. The expression of B-Cat and E-cadherins is higher in normal bladder cells than in cancer cells and the number of metastases and depth of invasion of the bladder cancer (20, 21).

Effect of B-Cat on thyrocyte transformation into a cancer cell is associated with the Wnt signaling pathway. Under normal conditions the level of B-Cat in the cytoplasm is kept low. Its increase causes migration of B-Cat to the cell nucleus which stimulates Wnt pathway to protein production. Mutations that perturb the function of B-Cat degradation lead to the stimulation of pathway protein production which contributes to the risk of thyroid cancer development (15). However, the correlation between B-Cat and the process of thyroid carcinogenesis which does not involve Wnt pathway, is of interest. Lu et al. (2012) pointed to the association between serum thyroid hormone levels in thyroid cancer and B-Cat-dependent E-cadherins. In a mouse model study they received an increased expression of the mentioned signaling pathway protein products (cyclin D2 protein) after stimulation of thyroid cells by hormones. This resulted in an increased incidence of follicular form of thyroid cancer in animals (ThrB (PV/PV) mouse mutants). B-Cat binding with metalloproteinase-2 (MMP2) affects the risk of metastases (22).

There are reports saying that the level of cellular B-Cat may be associated with thyroid hormone activity. There is indicated a completely Wnt-independent mechanism of B-Cat nuclear accumulation in follicular cells influenced by Thyrotropin (TSH) and insulin-like growth factor 1 (IGF-1). TSH and IGF-1, through the activation of protein kinase A and protein kinase B/Akt, phosphorylate B-Cat, which results in B-Cat release from E-cadherin. B-Cat nuclear accumulation causes reduction of the expression of Pax8, the main transcription factor involved in thyroid cell differentiation. The effect of Pax8 gene transcription reduction is associated with direct binding to B-Cat, which affects the inhibition of the process of follicular cell differentiation. One of the consequences of Pax8 gene inactivity is increased transcriptional activity on the sodium iodide symporter (NIS) gene (23). Its protein product is one of the most important regulatory factors required for thyroid cell physiology, being at the same time a biomarker of differentiated thyroid cancers (24).

Associations of B-Cat with thyroid transcription factor (TTF-1) play an important role in the transformation of thyrocytes into papillary carcinoma cells. TTF-1 detected, among others, in thyroid and lung cancer cells, is essential for proper thyroid cell differentiation (15). It has been shown that B-Cat is directly involved in the inhibition of TTF-1 expression in human thyroid cells. This provokes the appearance of mutant thyroid cells, thus becoming one of the factors of the development of papillary thyroid carcinoma (25).

In a study from the year 2000, there were examined immunohistochemically 206 specimens from patients with differentiated thyroid carcinoma. Positive staining for B-Cat was found in 67% of the papillary carcinoma specimens and in 55% of follicular carcinoma. Follicular carcinoma expressed more reduced staining pattern for B-Cat than papillary carcinoma. In this study, the reduction of cellular B-Cat expression was regarded as a negative prognostic factor in the assessment of the mortality of patients with thyroid cancer (26). The Canadian team examined the expression of B-Cat in 12 goiters operated on due to anaplastic thyroid cancer. Staining for B-Cat was compared in anaplastic carcinoma cells and in...
adjacent cells of differentiated cancers. Positive staining was demonstrated in 67% of differentiated cancer cells and in 50% of anaplastic carcinoma cells (27, 28, 29).

There are no studies related to normal values of plasma B-Cat concentration and to the relationship between the age of patients and B-Cat concentration (15, 30-33).

Our study demonstrated that patients with nodular goiter and papillary carcinoma had elevated ß-catenin levels as compared to those in the control group (tab. 2). Unfortunately, no differences were found in the concentrations of B-Cat between groups of patients with papillary carcinoma and nodular goiter. The fact that thyroid cancer in multinodular goiter coincides with benign thyroid gland lesions could be the cause.

Taking into account the obtained results, the possibility to design a diagnostic test which would use the investigated biomarker has been assessed. The dependence of the sensitivity and specificity on the position of the cutoff value is called the receiver operating characteristics – AUC. The bigger the area under the AUC is, the better the overall performance of the diagnostic test. The study included test, taking into account the value of the biomarker plasma concentration calculated acc. to the relationship of the concentrations of B-Cat. Most of the tests used in the diagnostics represent the diagnostic power expressed by AUC values between 0.8 and 0.95, whereas the test of AUC value > 0.7 is of significant diagnostic value. AUC values for test taking into account the use of the investigated biomarker was 0.721 for B-Cat (fig. 1).

**CONCLUSIONS**

On the basis of the above data it can be concluded that the decrease of B-Cat expression in thyrocyte cells speak for their transformation into neoplastic cells. This also reflects a possible transformation of differentiated thyroid cancer into the anaplastic form as well as the possibility of nodal metastases. However, plasma high B-Cat concentrations are associated with thyroid neoplastic disease. A diagnostic test taking into account plasma B-Cat concentrations allows the conclusion about the prognostic value of the investigated biomarker. ß-Catenin cannot be used as biomarker of papillary thyroid cancer because of coexisting tumor-like lesions in the thyroid gland.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>B-Cat (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GK / CG</td>
<td>48.8±4.45</td>
<td>67.9±11.55</td>
</tr>
<tr>
<td>RB / PTC</td>
<td>46.6±2.37</td>
<td>117.4±11.86</td>
</tr>
<tr>
<td>WG / NG</td>
<td>54.5±4.34</td>
<td>129.9±21.94</td>
</tr>
</tbody>
</table>

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