THE ROLE OF THE SIGNALING SYSTEM OF INSULIN-LIKE GROWTH FACTORS IN THE PROGRESS OF PAPILLAR THYROID CANCER

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INTRODUCTION. In recent decades, the incidence of thyroid cancer (TC) has been increasing faster than of any other cancer, while the mortality rate from TC remains stable [1, 2]. It should be emphasized that the increase in the incidence of TC is mainly caused by the increase in the papillary histological cases, while follicular or medullary subtypes are less often diagnosed [4]. Papillary thyroid cancer (PTC) accounts for 56% to 76% of all types of malignant tumors of the thyroid gland [5, 6]. This histological subtype of thyroid cancer is dangerous due to very gradual development, a sufficiently long latent period, which often causes its accidental diagnosis [7]; Ukraine is no exception to the increase in the incidence of thyroid cancer: more than 2,000 of cases are diagnosed annually [8]. All the mentioned raises concerns and makes the problem of diagnosis and treatment of malignant thyroid tumors very urgent.

The discovery of new molecular mechanisms of tumor growth is one of the promising individual approaches to diagnosis and treatment of patients with TC. Currently, the concept of a signaling pathway has been formed, which includes insulin-like growth factors (IGFs) – IGF-I, IGF-II and the IGF-I receptor [10]. 1FR-1 and IFR-II are mitogenic peptides highly homologous to each other and insulin. Regulation of this system at the cellular and tissue levels is created by six proteins that bind insulin-like growth factor (IGF) and proteinases that break them down. IGFs are synthesized in the liver and some other tissues under the influence of pituitary somatotropic hormone [11]. But they can also be produced by cells of different tumors and act as auto/paracrine mediators that mediate the growth, metastasis and anti-apoptotic responses of malignant cells [12].

A number of researchers have found out that aberrant IGF signaling is critical for the pathogenesis and progression of cancer. Thus, they have reported an increased expression of IGF ligands and receptors in tumors of the breast, lungs, pancreas, colon, prostate, ovaries, and thyroid gland, and noted the relationship with an unfavorable prognosis of the disease [13-15]. The role of IGF in apoptosis, transformation, invasion, and metastasis of tumor cells has also been proven. At the same time, the role of IGF in the circulating peripheral blood in patients with TC

has not been sufficiently studied.

OBJECTIVE: determine the level of insulin-like growth factors 1 and 2 in patients with PTC, depending on the main clinical and morphological parameters of the course of the disease.

MATERIALS AND METHODS. The study involved 70 patients with PTC, 48.9 ± 1.6 years old, who were treated at SO Grigoriev Institute of Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine in 2019-2020.

In accordance with the Ethical Principles for Medical Research Involving Human Subjects, Declaration of Helsinki, the World Medical Association (1964-2013), all patients were informed about the purpose and methods of the study and provided their written consent for the study. The research protocols were approved by the Bioethics and Deontology Committee of the SO Grigoriev Institute of Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine. All patients underwent a clinical examination after total thyroidectomy before special treatment (radioiodine therapy): ultrasound diagnosis of the neck and confirmation of PTC by pathomorphological examination of the postoperative material.

The patients were divided into 2 groups: group I – the main group (patients with PTC) – 60 patients, and group II – healthy patients – 10 patients.

Patients of group I (main) were distributed by stages of the tumor process following the TNM system according to the International TNM classification of malignant tumors [16]. There were 30 patients with stage $T_1N_oM_o$ (50.0% of cases), patients with tumor size from 2 to 4 cm – $T_2N_oM_o$ – 7 (12%), extrathyroidal spread (unilateral metastases to the lymph nodes – $T_1N_{1a}M_0$ – 8 cases (13.0%), $T_2N_{1a}M_0$ – 4 cases (7.0%), $T_3N_{1a}M_0$ – 5 cases (8.0 %). Bilateral involvement of cervical lymph nodes was as follows: $T_1N_{1b}M_0$ – 1 patient, $T_2N_{1b}M_0$ – 1 patient, $T_3N_{1b}M_0$ – 2 patients (total 4 patients – 7%). The distribution of patients of group I by size of the tumor and the presence or absence of cervical metastases is given in Table 1. That is, the vast majority of patients had the first and second stages of the disease (75%). None of the patients of this group had distant lung or bone metastases. The extrathyroidal spread

was observed in 21 (35%) patients. Moreover, unilateral and bilateral cervical lymph node involvement was observed in 17 (28.9%) and 4 (7%) patients, respectively. The second (control) group consisted of 10 healthy patients of the same age and sex.

All patients underwent anthropometric studies (height, weight) during the initial visit, with the following calculation of the body mass index (BMI) by formula:

$$BMI = \text{body weight} / \text{height}^2 \ (kg/m^2).$$

The BMI in both groups ranged from 18.2 to 53.5 (median 30.0).

Serum glucose was determined by the glucose oxidase method. Serum insulin was also determined (ELISA kit, Diagnostic System Laboratories); to assess insulin resistance, HOMA-IR was calculated by formula:

$$\text{HOMA-IR} = \text{fasting glucose (mmol/l)} \times \text{fasting insulin } (\mu U/ml) / 22.5.$$

The value > 2.7 indicated insulin resistance.

All patients were measured their serum IGF-1 and IGF-2 by enzyme immunoassay (ELISA) using a standard set of Human IGF-1 (Insulin-like Growth Factor 1) ELISA Kit and Human IGF-2 (Insulin-like Growth Factor 2) ELISA Kit (Elabscience Biotechnology Inc. USA). Measurements were performed with Immunochem-2100 (USA), a semi-automatic immunoenzyme analyzer.

Statistical analysis was performed using Statistica with non-parametric methods for small samples; in the absence of a normal distribution, median and quartile indicators (Me; Lq-Uq) are given in the series. Results were compared between groups using the Mann-Whitney test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION: Thirty patients (50%) were diagnosed with the first stage of the disease and the absence of signs of metastasis. Seven patients (12%) had the second stage of thyroid cancer, 2 patients had stage III, the process was localized only in the thyroid gland, that is, 39 patients of the main group had thyroid cancer localized only in the thyroid gland. Twenty-one patients (35% of cases) had PTC metastases in cervical lymph nodes. Unilateral involvement of cervical lymph nodes was found in 17 patients, which accounted for 28% of cases in the main group and almost 81% of cases with regional metastases. Four patients (7% of cases) had bilateral metastatic lesions of the cervical lymph nodes (19% of

the number of patients with extrathyroidal extension of the PTC).

The distribution of patients in the main group by clinical signs of the spread of the tumor was as follows: the analysis of serum IGF-1 and IGF-2 in patients with PTC (group I) after total thyroidectomy before radioiodine therapy showed that the level of IGF-1 in 63% of patients of group I was 2.7 times higher than in the control group, while 37% of cases had the same values as the control group. The level of IGF-2 in 85% of patients of group I was 3.1 times higher than in the control group, and 15% of patients of group I had this parameter within normal range. The data are shown in Table 1.

Table 1

Serum IGF-1 and IGF-2 in patients with PTC (Me; Lq-Uq)

Group	n, (%)	IGF-1, ng/ml	n, (%)	IGF-2, ng/ml
Control	10 (100)	21.5 (16.8-22.3)	10 (100)	102.5(95.1-112.2)
PTC patients	39 (65)	57.1*(53.1-64.5)	51 (85)	322.4* (310.6-341.5)
	21 (35)	19.1 (18.4-24.2)	9 (15)	96.1 (94.5-100.2)

*Note: * – reliability of differences in IGF-1 and IGF-2 content in patients of the main group compared to controls (Mann-Whitney test), p <0.05.*

Thus, a comparative analysis of the content of serum IGF-1 and IGF-2 in the PTC and practically healthy patients demonstrated in most cases high levels of IGF-1 and IGF-2 in PTC. According to the literature, the IGF signaling system plays an important role in carcinogenesis [16]. It has been proven that IGF-1 induces the activation of the MAP kinase pathway, which leads to the inhibition of apoptosis due to the deactivation of Bad, one of the pro-apoptotic proteins [17]. Increased expression of IGF ligands and receptors indicates an unfavorable prognosis of the disease. It has been established that the IGF system contributes to thyroid carcinogenesis through the IGF-2/IR-A autocrine loop, since IGF-2 activates the IR-A isoform that is expressed in neoplastic thyrocytes, thereby ensuring their proliferation and inhibition of apoptosis. Therefore, the increase in the levels of IGF-1 and IGF-2 in the blood serum of most PTC patients indicates the involvement of these factors in carcinogenesis [18].

Table 2 shows the content of serum IGF-1 and IGF-2 in PTC patients before special treatment after surgery, based on the clinical and morphological

characteristics of the disease.

Table 2

Serum IGF-1 and IGF-2 in PTC patients, based on the clinical and morphological characteristics of the disease.

Group	IGF-1, ng/ml		IGF-2, ng/ml	
	n	Median	n	Median
Control	10	21.5	10	102.5
PTC patients	39	57.1*	51	322.4*
Age				
Under 40	14	41.3*	14	299.2*
40-60	13	81,5* #	21	369.6*
>60	12	47.7*	16	283.7*
Sex				
Men	4	54.1*	7	403.2* #
Women	35	82.0* #	44	265.6*
BMI				
18.5-25	5	36.4	10	217.7*
25-30	14	65.6*	15	422.2* #
30-35	12	74.4* #	17	350.4*
35-40	7	30.8	8	217.7*
>40	1	33.0	1	262.0*
Insulin resistance (HOMA index)				
Up to 2.77	18	46.9*	29	287.5*
>2.77	21	66.2*	22	366.8*
Insulin				
Up to 24.9 mIU/ml	35	42.6*	46	279.5*
> 24.9 mIU/ml	4	180.2* #	5	708.6* #
Lymph node involvement (N criterion)				
N +	21	39.7*	33	252.5*
N-	39	86.7* #	18	446.7* #
TG				
Up to 10 ng/mL	27	57.6*	50	309.4*
10-50 ng/ml	9	44.3*	-	-
> 50 ng/ml	3	24.1	1	959.0* #

Table continuation 2

TgAb				
Up to 115 IU/ml	30	55.5*	40	316.2*
> 115 IU/ml	9	62.2*	11	344.4*

Notes: *1. * – reliability of differences relative to the control group, (Mann-Whitney test), p <0.05; 2. # – reliability of differences in groups relative to the distribution of clinical and morphological indicators (Mann-Whitney test), p < 0.05.*

Table 2 shows the uniform distribution of patients, mostly women (35 women versus 4 men), without metastases.

The levels of IGF-1 (63%) and IGF-2 (85%) were significantly higher in patients with PTC compared to the control group.

As Table 2 shows, there is no significant relationships between the levels of IGF-I and IGF-2 with the NOMA index, which characterizes insulin resistance, TG and TgAb. The relationship between the expression of IGF-1, IGF-2 and insulin was determined: with high insulin level > 24.9 mIU/ml, IGF-1 and IGF-2 increase 4.2 and 2.5 times, respectively.

The evaluation of the relationship between the content of IGF-1 and IGF-2 and the involvement of cervical lymph nodes showed that in the absence of lesions (N 0) an increase was by 2.2 and 1.8 times, respectively. Analysis of the distribution by body mass index shows that the IGF-1 and IGF-2 levels increase with BMI from 30 to 35 and from 25 to 30, respectively. It was found that IGF-1 was 1.5 times higher in women than in men, and IGF-2, on the contrary, was 1.5 times higher in men than in women. Patients aged 40 to 60 years also had a 1.7-fold increase in IGF-1, and a 1.2-fold increase in IGF-2.

Thus, the signaling system of insulin-like growth factors (IGF-1 and IGF-2) plays an important role in the development and progression of malignant tumors, in particular, PTC, so its components can be considered as potential diagnostic and prognostic markers of the disease and targets for anticancer therapy. Our data coincide with the earlier clinical data on the effect of signs of extrathyroidal spread of the thyroid gland on the course of thyroid cancer [19].

CONCLUSION.

1. The performed examination found a lesion within the thyroid gland in 65% of patients with PTC, extrathyroidal spread of the tumor in 35% of patients, a unilateral lesion of the regional cervical lymph nodes in 28% of cases, and bilateral – in 7% of cases.

2. In patients with papillary thyroid cancer, the content of IGF-I and IGF-2 in 63% and 85% patients, respectively, was probably higher compared to the control group.

3. We established the relationship between the content of IGF-1, IGF-2 and the high level of serum insulin as a proliferative factor in patients with PTC, which may indicate the aggressive potential of the disease.

4. The relationship between the expression of IGF-1, IGF-2 and insulin was

determined: with insulin level > 24.9 mIU/ml, IGF-1 and IGF-2 increase 4.2 and 2.5 times, respectively. The evaluation of the relationship between the content of IGF-1 and IGF-2 and the involvement of cervical lymph nodes showed that in the absence of lesions (N 0) an increase was by 2.2 and 1.8 times, respectively.

References:

1. Hershman JM. The Increased Incidence of Thyroid Cancer Is Worldwide. Clinical Thyroidolog. 2017; 29(1): 11-12. doi. https://doi.org/10.1089/ct.2017;29.11 -12
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016; 66(2): 115-132. doi. 10.3322/caac.21338
3. Lubitz CC, Kong CY, McMahon PM, Daniels GH, Chen Y, Economopoulos KP, et al. Annual financial impact of well-differentiated thyroid cancer care in the United States. Cancer. 2014; 120(9): 1345-1352. doi: 10.1002/СПСГ.28562
4. Liu Y, Su L, Xiao H. Review of Factors Related to the Thyroid Cancer Epidemic. Int J Endocrinol. 2017; 2017: 5308635. doi: 0.1155/2017/5308635
5. Lortet-Tieulent J, Franceschi S, Dal Maso L, Vaccarella S. Thyroid cancer "epidemic" also occurs in low- and middle-income countries. Int J Cancer. 2019; 144(9): 2082-2087. doi: 10.1002/ijc.31884
6. Hershman JM. Thyroid Cancer Incidence and Mortality Are Increasing. Clinical Thyroidology. 2017; 29: 221-223.
7. Dal Maso L, Panato C, Franceschi S, Serraino D, Buzzoni C, Busco S, et al. The impact of overdiagnosis on thyroid cancer epidemic in Italy, 1998-2012. Eur J Cancer. 2018; 94: 6-15. doi: 10.1016/j.ejca.2018.01.083
8. The main indicators of the endocrinology service of Ukraine in 2015. Endocrinology. 2016; 21(1): 1-38.
9. Chukur OO. Dynamics of morbidity and expansion of pathology of the thyroid gland among adult population of Ukraine. Bulletin of social hygiene and health care organization of Ukraine. 2019; 4: 19-25. doi: 0.11603/1681- 2786.2018.4.1002012
10. Bowers LW, Rossi EL, O'Flanagan CH, deGraffenried LA, Bursting SD. The role of the insulin/igf system in cancer: Lessons learned from clinical trials and the energy balance-cancer link. Front. Endocrinol (Lausanne). 2015; 6: 77. doi: 10.3389/fendo.2015.00077
11. Baxter, R.C. Igf binding proteins in cancer: Mechanistic and clinical insights. Flat. Rev. Cancer. 2014, 14, 329-341. doi: 10.3389/fendo.2015.00077
12. Weroha SJ, Haluska P. The insulin-like growth factor system in cancer. Endocrinol Metab Clin North Am. 2012; 41(2): 335-vi. doi: 10.1016/j.ecl.2012.04.014

13. CiampoIillo A, De Tullio C, Giorgino F. The igf-i/igf-i receptor pathway: Implications in thepathophysiology of thyroid cancer. Curr. Med. Chem. 2005; 12: 2881-2891.doi: 10.2174/092986705774454715
14. Vigneri PG, Tirro E, Pennisi MS., Massimino M., Stella S., Romano C., Manzella L. The insulin/igf system in colorectal cancer development and resistance to therapy. Front. Oncol. 2015; 5:230. doi: 10.3389/fonc.2015.00230
15. Simpson A, Petnga W, Macaulay VM, Weyer-Czemilofsky U, Bogenrieder T. Insulin-like growth factor (igf) pathway targeting in cancer: Role of the igf axis and opportunities for future combination studies. Target. Oncol. 2017; 12:571-597. doi: 10.1007/sl 1523-017-0514-5
16. Bowers LW, Rossi EL, O'Flanagan CH, deGraffenried LA, Bursting SD. The Role of the Insulin/IGF System in Cancer: Lessons Learned from Clinical Trials and the Energy Balance-Cancer Link. Front Endocrinol (Lausanne). 2015; 6: 77. doi: 10.3389/fendo.2015.00077
17. Zhang M, Liu J, Li M, Zhang S, L u Y, Liang Y, Zhao K, Li Y. Insulin-like growth factor 1/insulin-like growth factor 1 receptor signaling protects against cell apoptosis through the PI3K/AKT pathway in glioblastoma cells. ExpTherMed. 2018; 16(2): 1477-1482. doi: 10.3892/etm.2018.6336
18. Vella V, Malaguamera R. The Emerging Role of Insulin Receptor Isoforms in Thyroid Cancer: Clinical Implications and New Perspectives. Int J Mol Sci. 2018; 19(12): 3814.doi: 10.3390/ijms 19123814
19. Long-term experience of Grigoriev Institute for Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine in the treatment of thyroid cancer /
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