ORIGINAL ARTICLE

INSULIN-LIKE GROWTH FACTORS IN THE SERUM OF PATIENTS WITH PAPILLARY THYROID CANCER

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ABSTRACT

The aim is to study the level of insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2) in the blood serum of patients with papillary thyroid cancer, depending on the main clinical and morphological features of the disease.

Materials and methods: The material was the information about 60 patients with papillary thyroid cancer (group 1). In group 2 there were 10 patients without oncopathology. All patients underwent clinical examination after total thyroidectomy before special treatment (radioiodine therapy): ultrasound diagnosis of the neck, confirmed diagnosis of papillary thyroid cancer by morphological examination of operative material. All patients underwent anthropometric studies (height, weight), on the basis of which the body mass index (BMI) was calculated. The study program also included determination of the level of thyroid-stimulating hormone of the pituitary gland (TSH), thyroglobulin (TG), antibodies to thyroglobulin (AB-TG). It was also determined the serum glucose level. In order to assess insulin resistance, the HOMA-IR index was calculated. All patients were tested for serum IGF-1 and IGF-2.

Results: In the blood serum of patients with papillary thyroid cancer in 63% of patients the level of IGF-1 and in 85% – IGF-2 was probably higher than in the control group. There is a relationship between the level of IGF-1, IGF-2 and elevated level of proliferating factor – insulin in the serum of patients with papillary thyroid cancer. This may indicate an aggressive potential of the disease (i.e. clinical data on the prevalence of papillary thyroid cancer coincide with laboratory data). There was found a relationship between the expression of IGF-1, IGF-2 and insulin: at elevated levels of insulin> 24.9 μIU/ml, IGF-1 increases 4.2 times, and IGF-2 – 2.5 times. Evaluation of the relationship between the level of IGF-1 and IGF-2 and cervical lymph node involvement shows that in the absence of lesion (N0) there is an increase in these indicators by 2.2 and 1.8 times, respectively.

Conclusions: The signaling system of insulin-like growth factors (IGF-1 and IGF-2) plays an important role in the occurrence and progression of malignant tumors. It is especially true for papillary thyroid cancer, so its components can be considered as potential diagnostic and prognostic markers of the disease and targets for anticancer therapy.

KEY WORDS: insulin-like growth factor-1, insulin-like growth factor-2, blood serum, papillary thyroid cancer

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INTRODUCTION

Over the past few decades, there has been an increase in the incidence of thyroid cancer, and the rate of this growth is higher than any other cancers. However, mortality from thyroid cancer remains relatively stable [1, 2]. Although it is a less dangerous form of cancer against many other malignancies, the social and economic consequences of increasing incidence of thyroid cancer are obvious [3]. Interestingly, the increasing incidence of thyroid cancer is caused mainly by a growing number of cases of papillary histological variants. However, the growth of follicular or medullary subtypes is observed in a much smaller number of cases [4]. According to various data, the share of papillary thyroid cancer is from 56% to 76% of all types of malignant neoplasms of the thyroid gland [5, 6]. This histological subtype of thyroid neoplasms is dangerous due to the very gradual development, a long time latent course, which often causes accidental detection [7].

In Ukraine, we see an increasing incidence of thyroid cancer: more than 2 thousand new cases of this pathology are diagnosed annually [8]. The incidence of thyroid cancer was 4.3% of total endocrine morbidity in 2018 [9]. All this is worrying and makes the problem of detection and treatment of malignant thyroid tumors very relevant both in the world, and in Ukraine.

Identification of various molecular mechanisms of tumor growth is one of the promising approaches in the individualization of diagnosis and treatment of patients with thyroid cancer. Today, we have a concept of the formed signaling pathway which includes IGF-1, IGF-2 and IGF-1 receptor type [10]. IGF-1 and IGF-2 are mitogenic peptides, highly homologous to each other and insulin. This system is regulated at the cellular and tissue levels by six proteins that bind insulin-like growth factor and the proteinases that break them down. IGFs are synthesized in the liver and some other tissues under the influence of somatotropic hormone of the pituitary gland [11]. Nevertheless, they can also be synthesized by cells of various tumors and act as auto/paracrine mediators of the growth, metastasis and antiapoptotic responses of malignant cells [12].

To date, some studies have found that aberrant IGF signaling is critical in the pathogenesis and progression of cancer. It was found an increased expression of ligands and IGF receptors in tumors of the breast, lung, pancreas, colon, prostate, ovary and thyroid gland, while there was an association with an unfavorable prognosis [13-15]. Scientists have proved participation of IGF in apoptosis, transformation, invasion and metastasis of tumor cells. At the same time, the role of IGF in circulating peripheral blood in papillary thyroid cancer is insufficiently studied.

THE AIM

The aim was to study the level of IGF-1 and IGF-2 in the blood serum of patients with papillary thyroid cancer, depending on the main clinical and morphological features of the disease.

MATERIALS AND METHODS

The material was the information about 60 patients with papillary thyroid cancer aged 24 to 77 years (median 48.9±1.6), treated at State Organization "Grygoriev Institute for Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine" in the period 2019-2020.

According to the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as the object of study" (1964-2013), all patients were informed about the purpose, methods of research and gave their written consent to participate in the study. The research protocols were approved by the Committee on Bioethics and Deontology at State Organization "Grygoriev Institute for Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine".

All patients underwent clinical examination after total thyroidectomy before special treatment (radioiodine therapy): ultrasound diagnosis of the neck, confirmed diagnosis of papillary thyroid cancer by morphological examination of operative material.

All patients were divided into 2 groups: 1 – 60 patients with papillary thyroid cancer, 2 – 10 patients without oncopathology.

Patients in group 1 were distributed by stages of the tumor process according to the TNM system according to the International TNM classification of malignant neoplasms [16]. There were 30 patients (50%) with stage $T_1N_0M_0$, 7 patients (12%) with tumor size from 2 to 4 cm (stage $T_2N_0M_0$), patients with extrathyroid spread of thyroid cancer (metastasis to lymph nodes on one side – 8 patients (13%) with $T_1N_{1a}M_0$, 4 patients (7%) with $T_2N_{1a}M_0$, 5 patients (8%) with $T_3N_{1a}M_0$). Patients with lesions of the cervical lymph nodes on both sides distributed as follows: $T_1N_{1b}M_0$ – 1 patient, $T_2N_{1b}M_0$ – 1 patient, $T_3N_{1b}M_0$ – 2 patients (totally 4 patients (7%). Their distribution in group 1 by tumor size and presence or absence of cervical metastases is given in table I. That is, the majority of patients had the first and second stages of the disease (75%). No patient in this group had distant pulmonary or bone metastases. 21 (35%) patients had extrathyroid spread of thyroid cancer. Moreover, unilateral lesions of the

Table I. The distribution of patients with papillary thyroid cancer according to the TNM classification.

TNM	Absolute number	Relative number (%)
$T_1N_0M_0$	30	50
$T_2N_0M_0$	7	12
$T_3N_0M_0$	2	3
$T_4N_0M_0$	0	0
$T_1N_{1a}M_0$	8	13
$T_1N_{1b}M_0$	1	2
$T_2N_{1a}M_0$	4	7
$T_2N_{1b}M_0$	1	2
$T_3N_{1a}M_0$	5	8
$T_3N_{1b}M_0$	2	3
Total	60	100 %

Table II. Clinical and laboratory characteristics of the patients of group 1.

Parameter	Min	Max	M±m	Median
Weight, kg	50.0	142.0	81.2±2.4	79.5
Height, sm	150.0	191.0	164.0±1.1	162.0
BMI	18.2	53.5	30.3±0.9	30.0
Age (years)	24.0	77.0	48.9±1.6	47.5
AB-TG (IU/ml)	10.2	1189.0	126.0±33.1	21.0
TG (ng/ml)	0.1	144.3	41.2±18.7	10.0
TSH (μIU/ml)	4.8	98.4	60.8±3.3	56.4
Glucose (mmol/l)	4.0	8.0	5.3±0.1	5.2
Insulin (μIU/ml)	3.2	41.1	14.0±1.2	10.4
HOMA-IR index	0.6	10.2	3.4±0.3	2.6
C-peptide (ng/ml)	0.7	6.4	2.8±0.2	2.6
Glycated hemoglobin (%)	4.6	6.9	5.4±0.1	5.4
Blood calcium (mmol/l)	1.8	2.7	2.3±0.1	2.4

Table III. The level of IGF-1 and IGF-2 in the blood serum of patients of group 1 and 2.

Group number	n, (%)	IGF-1, ng/ml	n, (%)	IGF-2, ng/ml
1	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)
2	10 (100)	21.5 (16.8-22.3)	10 (100)	102,5 (95.1-112.2)

Note: * – significance of differences of parameter between group 2 and group 1

Table IV. The level of IGF-1 and IGF-2 in the blood serum of patients with papillary thyroid cancer depending on the clinical and morphological characteristics of the disease

Group number/Parameter	IGF-1, ng/ml		IGF-2, ng/ml	
	n	Median	n	Median
2	10	21.5	10	102.5
1	39	57.1*	51	322.4 *
Age (years)				
Till 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*
HOMA-IR index				
Till 2.77	18	46.9*	29	287.5*
> 2.77	21	66.2*	22	366.8*
Insulin				
Till 24,9 μIU/ml	35	42.6*	46	279.5*
> 24,9 μIU/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				
Till 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* #
AB-TG				
Till 115 IU/ml	30	55.5*	40	316.2*
> 115 IU/ml	9	62.2*	11	344.4*

Notes: * – significance of differences between group 2 and group 1; # – significance of differences in the group regarding the distribution of clinical and morphological parameters.

cervical lymph nodes were observed in 17 patients (28.9%), bilateral lesions – in 4 (7%) patients.

Group 2 consisted of 10 patients without cancer, who corresponded to the group 1 by age and sex.

All patients at the initial treatment underwent anthropometric studies (height, weight), on the basis of which the BMI was calculated according to the formula: BMI = body mass / height ^{2} (kg/м^2).

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

The study program also included determination of the level of TSH, TG, AB-TG (test system BRAHMS Henning Berlin GMBH, Germany). Serum glucose level was determined by glucose oxidase method.

The author also determined the level of insulin in the blood serum (test system from Diagnostic System Laboratories, USA). In order to assess insulin resistance, the HOMA-IR index was calculated according to the formula:

HOMA-IR = fasting glucose (nmol/L) × fasting insulin (microU/L) / 22.5.

Index value> 2.7 indicated the presence of insulin resistance.

The obtained clinical data are shown in table II.

All patients were tested for serum IGF-1 and IGF-2. An enzyme-linked immunosorbent analysis (ELISA) was performed using the standard set of reagents "Human IGF-1 ELISA Kit" and "Human IGF-2 ELISA Kit" (Elabscience Biotechnology Inc., USA). Measurements were performed with a semi-automatic enzyme-linked immunosorbent analyzer "Immunochem-2100" (USA).

All patients were tested for serum IGF-1 and IGF-2 by enzyme-linked immunosorbent assay (ELISA) using the standard set of reagents "Human IGF-1 (Insulin-like Growth Factor 1) ELISA Kit" and "Human IGF-2 -like Growth Factor 2) ELISA Kit "by Elabscience Biotechnology Inc. USA ». Measurements were performed with a semi-automatic enzyme-linked immunosorbent assay "Immunochem-2100" (USA).

Statistical analysis of the data was performed using the statistical software package "Statistica" by non-parametric methods for small samples. In the absence of a normal distribution, the medians and quartiles (Me, Lq-Uq) were given in a row. The results were compared between groups, using the Mann-Whitney test. Discrepancies were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

According to clinical data, 30 patients (50%) had the first stage of the disease and no signs of metastasis. 7 patients (12%) had the second stage of papillary thyroid cancer, 2 patients (3%) – stage III. Thus, in 39 patients (75%) of group 1 papillary thyroid cancer localized only in thyroid gland without metastasis. 21 patients (35%) of group 1 had metastases of the cervical lymph nodes. Unilateral lesions of the cervical lymph nodes were found in 17 patients (28%) and 4 patients (7%) had bilateral metastatic lesions of the cervical lymph nodes.

Analysis of IGF-1 and IGF-2 levels in the serum of patients of group 1 and 2 showed that IGF-1 in 65% of patients in group I was 2.7 times higher than in group 2, in 35% of cases – was at the level of control values. The level of IGF-2 in 85% of patients in group 1 was 3.1 times higher than in group 1, while in 15% of patients in group 1 this parameter did not differ from the norm. The data are shown in table III.

Thus, a comparative analysis of IGF-1 and IGF-2 level in the blood serum of patients with papillary thyroid cancer (group 1) and almost healthy individuals (group 2) showed in most cases an increased level of IGF-1 and IGF-2 in patients with papillary thyroid cancer. According to the literature, the IGF signaling system plays an important role in carcinogenesis [16]. IGF-1 has proved to induce mitogen-activated protein kinase pathway, leading to inhibition of apoptosis through inactivation of one of the proapoptotic proteins, Bad [17]. An increased expression of IGF ligands and receptors indicates an unfavorable prognosis of the disease. The IGF system has been shown to contribute to thyroid carcinogenesis through the IGF-2/IR-A autocrine loop because IGF-2 activates the IR-A isoform, expressed in neoplastic thyrocytes, thereby ensuring their proliferation and inhibition of apoptosis. Thus, increasing level of IGF-1 and IGF-2 in the blood serum of most patients with papillary thyroid cancer indicates the role of these factors in the processes of carcinogenesis [18].

The level of IGF-1 and IGF-2 in the blood serum of patients with papillary thyroid cancer before special treatment after surgery, depending on the clinical and morphological characteristics of the disease are given in table IV.

As one can see from table 4, distribution of patients without metastases was homogeneous, mostly women (35 women vs. 4 men).

The levels of IGF-1 (63%) and IGF-2 (85%) were significantly higher in patients with papillary thyroid cancer compared with the group 2.

It is clear from table 4 that significant relationships between IGF-1 and IGF-2 levels with the HOMA-IR index, which characterizes insulin resistance, TG with AB-TG did not reveal. The author determined relationship between the expression of IGF-1, IGF-2 and insulin: at elevated levels of insulin > 24.9 μIU/ml, IGF-1 increases 4.2 times, and IGF-2 – 2.5 times.

Evaluation of the relationship between the level of IGF-1 and IGF-2 and cervical lymph node involvement showed that in the absence of lesion (N 0) there was an increase of these indicators by 2.2 and 1.8 times, respectively. Analysis of the distribution by BMI showed that the content of IGF-1 increased with BMI from 30 to 35, and IGF-2 – with BMI from 25 to 30. The author has found that IGF-1 was 1.5 times higher in women than in men, and IGF-2, in contrast, 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.

Thereby, the signaling system of insulin-like growth factors (IGF-1 and IGF-2) plays an important role in the occurrence and progression of malignant tumors. It is especially true for papillary thyroid cancer, so its components can be considered as potential diagnostic and prognostic markers of the disease and targets for anticancer therapy. Our data coincide with clinical data about the influence of extrathyroid spread of papillary thyroid cancer on the course of thyroid cancer obtained earlier [19, 20].

CONCLUSIONS

1. In 65% of patients with papillary thyroid cancer there was a lesion only within the thyroid gland, in 35% of patients the tumor process spread beyond the organ, in 28% of cases there were unilateral lesions of regional cervical lymph nodes, in 7% of cases – bilateral metastatic lesions.
2. In the blood serum of patients with papillary thyroid cancer in 63% of patients the level of IGF-1 and in 85% – IGF-2 was probably higher than in the control group.
3. There is a relationship between the level of IGF-1, IGF-2 and elevated level of proliferating factor – insulin in the serum of patients with papillary thyroid cancer. This may indicate an aggressive potential of the disease (i.e. clinical data on the prevalence of papillary thyroid cancer coincide with laboratory data).
4. There was found a relationship between the expression of IGF-1, IGF-2 and insulin: at elevated levels of insulin> 24.9 μIU/ml, IGF-1 increases 4.2 times, and IGF-2 – 2.5 times. Evaluation of the relationship between the level of IGF-1 and IGF-2 and cervical lymph node involvement shows that in the absence of lesion (N0) there is an increase in these indicators by 2.2 and 1.8 times, respectively.

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Conflict of interest:

The Authors declare no conflict of interest.

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