Elevated serum concentrations of IGF-1 and IGF-1R in patients with thyroid cancers

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Background. The rising incidence of thyroid cancer observed in the last few decades requires an improvement in diagnostic tools and management techniques for patients with thyroid nodules.

Aims. The aim of this study was to assess serum concentrations of IGF-1 and IGF-1R in patients diagnosed with thyroid cancers.

Methods. 36 patients diagnosed with papillary thyroid cancer (PTC), 11 subjects with follicular thyroid cancer (FTC), 9 patients with anaplastic thyroid cancer (ATC) and 19 subjects with multinodular nontoxic goiter (MNG) were enrolled to the study. The control group (CG) consisted of 20 healthy volunteers. Blood samples were collected one day before surgery. Serum IGF-1 and IGF-1R concentrations were measured using specific ELISA methods.

Results. Significantly higher concentrations of IGF-1 were found in patients with PTC as compared with controls but not that obtained from subjects diagnosed with MNG. The concentration of IGF-1R was significantly elevated in subjects with PTC and ATC as compared with healthy volunteers. Similarly, patients diagnosed with PTC or ATC presented significantly higher serum concentration of IGF-1R in comparison to the MNG group.

Conclusions. Our results show that the IGF-1 – IGF-1R axis plays a significant role in the development of PTC and ATC and imply that serum concentrations of both cytokines may be considered as additional markers for the differentiation of malignancies during the preoperative diagnosis of patients with thyroid gland tumors. These results indicate that IGF-1R serum concentrations allow us to differentiate between MNG and PTC or ATC. Moreover IGF-1R serum values appear to be better predictor of PTC and ATC than IGF-1 concentrations.

Key words: thyroid cancer, IGF-1, IGF-1R

INTRODUCTION

Thyroid cancer (TC) is the most common endocrine malignancy with a rapidly rising incidence rate that has been observed continuously all over the world over the last few decades. Recently ~1.5% of new cancer cases in Europe have been diagnosed as TC (ref.1). Currently, according to the National Cancer Institute Surveillance, Epidemiology, and End Results Program, TC accounts for 3.4% of all cancers diagnosed yearly and, in 2030, it is estimated to be the fourth leading cancer diagnosis in United States. Much of this rise appears to be the result of progress in diagnostics, e.g. the increased use of thyroid ultrasound, which can detect small thyroid nodules that might not otherwise have been found in the past (www.cancer.org). Thus an improvement in reliable diagnostics and management techniques for patients diagnosed with certain types of TC is required in order to avoid overdiagnosis and overtreatment in low-risk patients and underdiagnosis and undertreatment in high-risk patients.

Histopathological classification differentiates TC derived from follicular thyroid cells as the most frequent papillary thyroid cancer (PTC), followed by follicular thyroid cancer (FTC), poorly differentiated thyroid cancer and rare anaplastic thyroid cancer (ATC). PTC and FTC subtypes are together classified as differentiated thyroid cancers (DTC) with good prognosis, while ATC is a lethal malignancy. Thus, histopathological diagnosis of TC subtypes together with TNM staging and molecular markers (including BRAF and RAS point mutations; RET/PTC and PAX8/PPARY genes rearrangements; MAPK; PI3K; p53; Wnt-beta catenin; HIF1α and NF-kappaB signaling pathways; and microRNA profiles) are critical for patient management and prognosis.

The recent search for preoperative serum biomarkers for thyroid carcinoma has resulted in the establishment...
of a preoperative neutrophil-to-lymphocyte ratio that closely relates to the stage of PTC (ref.9), preoperative thyroglobulin antibodies as potential marker for PTC in patients with indeterminate thyroid nodules10, serum SERPINE2/Protease Nexin-1 and Secretory Leukocyte Protease Inhibitor (SLPI) concentrations as preoperative PTC biomarkers11, circulating miRNAs as a non-invasive biomarker for the diagnosis of PTC (ref.12) and preoperative serum thyroglobulin (Tg) and changes in serum Tg during thyroid stimulating hormone (TSH) suppression as independent predictors of FTC in thyroid nodules with a cytological diagnosis of follicular lesion13. Nevertheless, continuous search for specific growth factors involved in the pathogenesis and progression of differentiated and anaplastic thyroid cancers still remains of utmost importance for improving preoperative diagnostics and for designing new therapeutic approaches to dealing with TC.

The insulin-like growth factors (IGFs) family, comprised of insulin and two natural growth hormones, IGF-1 and IGF-2, is crucially involved in cellular growth, survival and development. IGFs directly regulate cellular functions by interacting with specific cell surface receptors, mainly type I (IGF-1R). IGF-1R belongs to the family of tyrosine kinase growth factor receptors which are involved in cell transformation and protection of transformed phenotypes14. It has been proved that the IGF1 – IGF-1R axis plays an essential role in cancer pathogenesis by suppressing apoptosis and promoting cell cycle progression, angiogenesis, and metastatic activities in various cancers14. This is achieved by direct activation of the PI3K/Akt signaling pathway initiated by IGF-1 – IGF-1R, leading to stimulation of mitogenesis, cell cycle progression, and protection against apoptotic stresses. The IGF-1 axis activates some other signaling pathways involved in cells growth such as MAPK and mTOR. Alterations of the IGF-IGFR system in lung cancer, gastrointestinal neoplasms (colorectal, gastric, pancreatic, and hepatocellular cancers), female and male reproductive system neoplasms (breast, ovarian, endometrial, cervical, and prostate cancers), skin malignancies (basal cell carcinoma and melanoma malignum), head and neck cancers, bone and soft tissue cancers, hematologic malignancies, central nervous system neoplasms, urinary tract neoplasms have been described (for review see Kasprzak et al.15). Thus, the components of the IGF system are first cytokines considered as diagnostic serous biomarkers and prognostic factors for various cancers, as well as targets for modern anti-tumor therapies14,15. The proved presence of IGF-1 in normal and thyroid cancer tissues with significantly higher values in malignant tissues than in normal tissues as well as the overexpression of IGF-1 receptors found in both thyroid cancer cell lines and specimens16 resulted in the conception that the IGF system may be involved in thyroid cancer growth17.

Regardless of the significant advances in TC preoperative diagnostics, including certain molecular and serum markers characteristic for DTC and some progress in understanding pathogenesis of ATC, there is still a great need for a better understanding of TC pathogenesis and improvement in preoperative diagnostics. Moreover, although the malignancy rate of incidental thyroid nodules in healthy populations is quite low (~5%), it depends on patients’ selection and detection technique: in lesions found in FDG-PET the malignancy rate reaches 33-35% (ref.18), and in patients with non-thyroidal malignancies managed by USG or PET/CT, PTC proportion in identified nodules was 3.4% or 8.9% respectively19. Thus, the improvement in diagnostics of thyroid nodules and identification of those in need of surgical treatment is of utmost importance. Therefore, the aim of the present study was to identify whether IGF-1 and IGF-1R serum concentrations might be considered additional prognostic markers of malignancy in patients diagnosed with thyroid gland nodules.

### MATERIAL AND METHODS

#### Study design and patient characteristics

Seventy-five euthyroid patients aged from 18 to 75 years (53.72 ± 12.62) (mean ± standard error of the mean) that underwent surgery in the Clinic of Endocrinological and General Surgery, Copernicus Memorial Hospital, Lodz, Poland were enrolled in the study. The examined group was composed of 36 subjects diagnosed with PTC (PTC group), 11 with FTC, 9 with ATC and 19 with multinodular nontoxic goiter (MNG). Selected cases were diagnosed by fine-needle biopsy and confirmed by postoperative histopathologic examination. Other thyroid gland pathologies were excluded on the basis of familial and clinical history, clinical examination, ultrasonography and thyroid function tests (aTPO, aTG, TSH, fT3, fT4 and calcitonin serum concentrations). The control group (CG) included 20 healthy, age-matched volunteers with no history of any thyroid disease confirmed by clinical and hormonal tests, thyroid

| Group | Number of patients (Gender F/M) | Age (years) Mean ± SEM |
|-------|---------------------------------|------------------------|
| Control | 20 (10/10) | 55.15 ± 9.80 |
| MNG | 19 (10/9) | 54.71 ± 10.23 |
| PTC | 36 (20/16) | 52.73 ± 11.20 |
| FTC | 11 (6/5) | 52.42 ± 9.11 |
| ATC | 9 (4/5) | 57.12 ± 12.36 |

PTC = papillary thyroid cancer, FTC = follicular thyroid cancer, ATC = anaplastic thyroid cancer, MNG = multinodular nontoxic goiter, C = healthy controls
ultrasound scan, and the presence of thyroid autoantibodies. Demographic and clinical characteristics of the examined groups and healthy controls are presented in Table 1. All patients diagnosed with thyroid cancer (PTC, FTC or ATC) were treated with total thyroidectomy, and therapeutic neck dissection was performed with standard indications. The MNG patients group was treated with bilateral subtotal thyroidectomy. Histopathological diagnosis and clinical staging of patients included in the study is presented in Table 2. The multinodular nontoxic goiter group was comprised of 19 cases with benign postoperative histopathological diagnoses, including 8 follicular adenomas and 11 adenomatous nodules.

The project was approved by the Bioethics Committee of the Medical University of Lodz.

Measurements of IGF-1 and IGF-1R serum levels by ELISA

Blood samples were collected from the antecubital vein between 7:00 and 8:00 am after an overnight fast, one day before surgery. Blood samples were processed within one hour after collection, and the serum was aliquoted and stored at -80 °C until analysis. Determination of IGF-1 (Mediagnost GmbH) and IGF-1R (Shanghai Sunred Biological Technology Ltd.) concentrations were evaluated using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer’s instructions. All measurements were taken in duplicate and averaged.

Statistical analysis

Results were presented as mean ± standard error of the mean (SEM) in the figures and text and as mean ± standard deviation (SD) in the table. The Shapiro-Wilk test was applied to analyze the data distribution. ANOVA followed by Fisher’s protected Least Significant Difference was used to calculate differences between investigated groups; P<0.05 was considered significant. All statistical analyses were performed using StatSoft statistical software v.10.0. (Statistica PL).

RESULTS

The quantitative determination of the IGF-1 and IGF-1R concentration in the serum and the statistical evaluation of these results are presented in Fig. 1. and 2. and Table 3. The mean serum concentration of IGF-1 in PTC patients (300.6 ± 82.2 ng/mL) was significantly higher than that obtained in controls (216.0 ± 27.6 ng/mL; P<0.01) (Fig. 1, Table 3). The mean IGF-1/R serum concentration was significantly higher in patients diagnosed with PTC (39.7 ± 22.8 ng/mL) than in healthy volunteers (16.7 ± 3.0 ng/mL; P<0.05) or in MNG subjects (18.4 ± 2.5 ng/mL; P<0.05) (Fig. 2, Table 3).

The mean serum concentration of IGF-1 in FTC (265.3 ± 33.1 ng/mL), ATC (190.3 ± 17.9 ng/mL) and MNG (242.1 ± 29.6 ng/mL) patients did not significantly differ from that of the control group (216.0 ± 27.6 ng/mL). Similarly, the mean serum concentration of IGF-1R in FTC patients (29.3 ± 3.0 ng/mL) did not significantly differ from that of the healthy volunteers (16.7 ± 3.0 ng/mL) or the MNG group (18.4 ± 2.5 ng/mL).

Table 2. Histopathological diagnosis and clinical staging of thyroid cancer patients recommended by the 2010 TNM system edition by the Union for International Cancer Control.

| I  | II  | III | IVA |
|----|-----|-----|-----|
| PTC (n=36) | 12  | 10  | 2   |
| FTC (n=11) | 5   | 1   | 1   |
| ATC (n=9) | 0   | 4   | 5   |

PTC – papillary thyroid cancer, FTC – follicular thyroid cancer, ATC – anaplastic thyroid cancer

Table 3. Mean serum concentration ± SD of IGF-1 (ng/mL) and IGF-1R (ng/mL) in patients diagnosed with papillary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, multinodular goiter and healthy volunteers.

| Mean±SD | Control | MNG | PTC | FTC | ATC |
|---------|---------|-----|-----|-----|-----|
| IGF-1 [ng/mL] | 216.0±123.4 | 242.1±129.0 | 300.6±82.2 | 265.3±104.8 | 190.3±53.7 |
| IGF-1R [ng/mL] | 16.7±13.4 | 18.4±10.9 | 39.7±22.8 | 29.3±10.1 | 36.3±24.6 |

C – healthy controls, MNG – multinodular nontoxic goiter, PTC – papillary thyroid cancer, FTC – follicular thyroid cancer, ATC – anaplastic thyroid cancer
Fig. 1. Mean serum concentration of IGF-1 (ng/mL) in patients diagnosed with papillary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, multinodular goiter and healthy volunteers. Bars represent mean ± SEM
C – healthy controls, PTC – papillary thyroid cancer, MNG – multinodular nontoxic goiter, FTC – follicular thyroid cancer, ATC – anaplastic thyroid cancer

Fig. 2. Mean serum concentration of IGF-1R (ng/mL) in patients diagnosed with papillary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, multinodular goiter and healthy volunteers. Bars represent mean ± SEM
C – healthy controls, PTC – papillary thyroid cancer, MNG – multinodular nontoxic goiter, FTC – follicular thyroid cancer, ATC – anaplastic thyroid cancer

DISCUSSION

Insulin and IGF-1 have been shown to be important growth factors that, together with TSH, interact in the regulation of thyroid cells proliferation and differentiation\textsuperscript{20}. The expression of IGF-1 and IGF-1R in thyroid malignancy has been documented\textsuperscript{21,22}, but the role of the IGF-1-IGF-1R system in TC, especially in DTC, has not been widely explored. IGF-1 and IGF-1R or IGF-1R mRNA were measured in thyroid cancer cell lines and tissue specimens from thyroid epithelial tumours showing overexpression of IGF-1 and IGF-1 receptors in malignant tissues. However, data concerning in vivo IGF-1 expression in TC and its relevance as a prognostic factor are limited\textsuperscript{20}. 

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IGF-1 and IGF-1R were detected in both PTC and FTC subtypes in children and adolescents. IGF-1 and IGF-1R expression was found more often in PTC than in normal surrounding thyroid tissue and intense IGF-1R staining was connected with more aggressive clinical features. Although the relationship between TC and nodular goiter is still debated, IGF-1 expression in tissues obtained from patients who had undergone total thyroidectomy for nodular thyroidal disease was higher in benign nodules and papillary carcinomas than in extranodular healthy tissues in the same patients and was higher in papillary carcinomas than in benign nodules. Interestingly, IGF-1 levels in tissue were higher in nodules than in healthy tissues in patients with papillary cancer. In our study, IGF-1R serum concentration in patients diagnosed with MNG did not significantly differ from values obtained in healthy subjects. In other studies on thyroid nodules, IGF-1 and IGF-1R expression as well as IGF-1 mRNA and IGF-1R mRNA expression were shown to be significantly higher in nodular goiters, follicular adenomas, and PTC than in normal thyroid tissues. PTC tissues showed significantly higher IGF-1 and IGF-1R protein and mRNA expression compared with follicular adenomas and nodular goiters. These findings indicate that IGF-1 plays a role in the development of benign and malignant thyroid nodules and suggest a link between IGF-1 and papillary cancer that may be equally or more intense than that between IGF-1 and benign nodules. Other types of thyroid cancers do not seem to have such a clear correlation with the tissue levels of IGF-1 as that postulated in the case of patients diagnosed with PTC. In fact, Chakravarty et al. reported significantly higher expression of phosphorylated IGF-1R in highly differentiated than in poorly differentiated thyroid cancers. Our results showed higher serum concentrations of IGF-1 in PTC patients than in controls and higher serum IGF-1R concentrations in those diagnosed with PTC, than in healthy volunteers and multinodular nontoxic goiter subjects. Our data is coherent with the findings from samples of healthy thyroid, nodular goiters and PTC tissues and support the role of the IGF-1-IGF-1R pathway dysfunction in pathogenesis of DTC. In fact, increased expression of phosphorylated p70S6 and Akt, which both are kinases phosphorylated and activated by growth factors including IGF-1, found in papillary thyroid cancer tissues further supports the role of the IGF-1 axis in PTC pathogenesis and development. However, in contrast to the mentioned studies on poorly differentiated TC tissues samples, we observed a higher IGF-1R serum concentration in the group of ATC patients than in controls or MNG subjects. It must be stressed that our results concerning higher serum IGF-1R concentrations in anaplastic cancer patients in in vivo conditions are supported by the studies of Wang et al. who demonstrated IGF-1R expression on ATC tissue samples and the inhibitory effect of IGF-1 antibody on cell proliferation and angiogenesis as well as its proapoptotic activity in an orthotopic nude mouse model of anaplastic thyroid cancer in vivo.

In addition, a positive association between IGF-1 concentrations and risk of differentiated thyroid cancer was documented in the prospective multicenter study with a group of 345 incident cases of DTC who were individually matched to 735 controls by study center, sex and age, date, time, fasting status at blood collection, follow-up duration, and menopausal status, use of exogenous hormones, and phase of menstrual cycle at blood collection for women. This can be confirmed by uncontrolled hyperactivity of the GH-IGF-1 axis in the development of PTC rather than the BRAFV600E mutation in acromegalic patients and by the observation that thyroid cancer is the most common cancer associated with acromegaly.

In contrast to PTC diagnosed patients, the lack of significant changes in IGF-1 or IGF-1R serum concentrations in FTC subjects observed in our study was in agreement with Schmidt et al. In our results expand and complement the mentioned studies on DTC and additionally suggest the role of the IGF-1-IGF-1R system in anaplastic thyroid cancer development and progression as well. The latter is particularly interesting since TSH is known to be the major growth factor for differentiated thyroid cancer, while TSH independent cancer growth and metastatic spread become important in cases with loss of differentiation. This means the efficacy of TSH suppression in poorly differentiated or undifferentiated TCs is limited. Our results indicate the potential role of the IGF-1 - IGF-1R system in those cases and might suggest the beneficial blockade of IGF-1R in the management of patients diagnosed with ATC. This can be supported by the aforementioned study of Wang et al. who demonstrated the inhibition of cell proliferation and antiangiogenic and proapoptotic activity of the IGF-1 antibody on ATC animal model in vivo. Moreover, as it was shown in thyroid cell cultures, the mitogenic effects of TSH may be insignificant without other growth factors, although they are greatly enhanced by the presence of insulin or IGF-1.

The studies concerning serum biomarkers of thyroid cancer make evident the fact that development of TC is a complex process that involves multiple metabolic and growth factors pathways’ dysregulation. In our previous studies on thyroid cancer, PTC, FTC and ATC patients had significantly lower concentrations of βKlotho as compared with the MNG group and controls. Interestingly, secreted Klotho is a circulating protein with anti-aging and anti-oncogenic properties that modulates the activity of multiple growth factors receptors, including the IGF-1R axis and is downregulated in several neoplasms.

Together with the results of the present paper, the interplay between circulating βKlotho and IGF-1R axis in the development of PTC and ATC might be considered.

CONCLUSIONS

Our findings support the role of the IGF-1 – IGF-1R axis in the development of papillary cancer and suggest the involvement of overexpression of this pathway in anaplastic cancer as well. These results indicate that only IGF-1R values are able significantly differentiate between MNG and PTC or ATC (but not FTC) in this study with respect to lim-
mented number of patients. Moreover it seems, that IGF-1R is a better predictor of PTC and ATC than IGF-1. Thus IGF-1R serum concentration can be considered as additional biomarker for improving preoperative diagnosis of patients with thyroid nodules and with special value for papillary and anaplastic thyroid cancers diagnosis.

ABBREVIATIONS

ATC, Anaplastic thyroid cancer; CG, Control group; DTC, Differenitated thyroid cancers; FTC, Follicular thyroid cancer; GH, Growth hormone; IGF-1, Insulin-like growth factor type 1; IGF-1R, Insulin-like growth factor type 1 Receptor; IGF-2, Insulin-like growth factor type 2; IGFS, Insulin-like growth factors family; MAPK, Mitogen-activated protein kinases; MNG, Multinodular nontoxic goiter; mTOR, Mammalian target of rapamycin kinase; PTC, Papillary thyroid cancer; SLPI, Secretory proteinase Inhibitor; TC, Thyroid cancer; Tg, Thyroglobulin; TSH, Thyroid stimulating hormone.

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