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Serum endostatin levels in patients with metastatic and non-metastatic well-differentiated thyroid cancer

Ocena stężenia endostatyny w surowicy krwi chorych z obecnością i bez przerzutów zróżnicowanego raka tarczycy

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Abstract

Introduction: The growth of a tumour is limited by its neovascularisation. Angiogenesis is dependent on a dynamic balance between its activators and inhibitors. One of the most important antiangiogenic factors is endostatin.
The aim of the study was to assess the usefulness of serum endostatin levels as a potential marker of metastases of well-differentiated thyroid cancer, and to estimate the effect of endogenous TSH stimulation on serum endostatin levels.
Material and methods: The study group consisted of 68 patients with differentiated thyroid cancer. We provided a cross-sectional analysis of the study group divided into patients with distant and/or locoregional metastases and patients with remission, and compared it with serum endostatin levels of healthy volunteers. Serum endostatin concentration was measured by ELISA kits (R&D Systems).
Results: Median endostatin concentration was significantly higher in patients with distant metastases than in patients with remission (141.95 *v.* 105.345 ng/ml, $p < 0.05$). This was not observed in patients with locoregional metastases. Serum endostatin levels were significantly higher in the study group, including patients with remission, than in the control group of healthy volunteers (remission *v.* healthy median 105.3 *v.* 88.1 ng/ml, $p < 0.05$). During endogenous TSH stimulation, endostatin levels significantly decreased (122.94 *v.* 93.60 ng/ml, $p < 0.05$).
Conclusions: The study indicates that endogenous TSH stimulation plays a role in the regulation of endostatin secretion. Although median serum endostatin levels are higher in patients with distant metastases than in patients with remission, its clinical usefulness is limited due to the overlapping data between the study groups. **(Pol J Endocrinol 2010; 61 (1): 7–12)**

Key words: *endostatin, thyroid cancer, metastases, TSH stimulation*

Streszczenie

Wstęp: Wzrost nowotworów jest zależny od stopnia ich unaczynienia. Angiogeneza jest zależna od dynamicznej równowagi między jej aktywatorami i inhibitorami. Jednym z najważniejszych czynników antyangiogennych jest endostatyna.
Celem pracy była ocena przydatności pomiaru stężenia endostatyny w surowicy krwi, jako potencjalnego wskaźnika obecności przerzutów zróżnicowanego raka tarczycy oraz oszacowanie wpływu endogennej stymulacji hormonu tyreotropowego (TSH, *thyroid stimulating hormone*) na stężenie endostatyny w surowicy.
Materiał i metody: Grupa badana składała się z 68 pacjentow ze zróżnicowanym rakiem tarczycy. Przeprowadzono analizę przekrojową stężenia endostatyny w surowicy krwi pacjentów, którzy w wyniku zastosowanego leczenia uzyskali remisję w porównaniu z jej stężeniem u chorych z przerzutami lokoregionalnymi i odleglymi. Stężenie endostatyny oznaczono za pomocą testów ELISA R&D Systems.
Wyniki: Średnie stężenie endostatyny było znamiennie wyższe u chorych z przerzutami odleglymi raka tarczycy niż u pacjentów w remisji (141,95 *v.* 105,345 ng/ml, $p < 0,05$). Stężenie endostatyny nie różniło się istotnie między chorymi z przerzutami lokoregionalnymi i pacjentami w remisji.
Stężenia endostatyny były znamiennie wyższe w całej grupie badanej, w tym u pacjentów w remisji, w porównaniu ze stężeniem stwierdzonym u zdrowych ochotnikow (mediana remisja *v.* zdrowi 105,3 *v.* 88,1 ng/ml, $p < 0,05$). W czasie endogennej stymulacji TSH stężenie endostatyny znamiennie się zmniejszyło (122,94 *v.* 93,60 ng/ml, $p < 0,05$).
Wnioski: Endogenna stymulacja TSH bierze udział w regulacji wydzielnia endostatyny. Stężenie endostatyny jest znamiennie wyższe u chorych z przerzutami odległymi raka tarczycy w porównaniu z pacjentami w remisji. Obserwacja ta ma jednak ograniczone znaczenie kliniczne, ponieważ nie można ustalić wartości progowej stężenia endostatyny wskazującej na obecność przerzutów odleglych.
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Słowa kluczowe: *endostatyna, rak tarczycy, przerzuty, stymulacja TSH*

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Introduction

Angiogenesis is an important process involved in the growth of normal and neoplastic tissues [1]. New blood vessel formation depends on a dynamic balance between the activators and inhibitors of angiogenesis. The factors involved in this process are potential markers of tumour spread and consequently are possible targets for therapy. The activated endothelial cells required for angiogenesis are theoretically free of drug resistance, in contrast to the cancer cells rapidly changing due to genomic instability.

One of the most potent inhibitors of neoangiogenesis is endostatin — a 20 kDa internal fragment of the carboxy terminus of collagen XVII. This small protein acts widely in the regulation of the angiogenesis — mainly suppressing pathological angiogenesis and having little or no activity against wound healing or reproduction [1]. There is an evidence that 12% of all human genes are significantly regulated in human microvascular endothelial cells exposed to endostatin [2, 3].

Therefore, endostatin represents a broad-spectrum inhibitor of angiogenesis which could be used in the therapy of cancer. In fact, endostatin has been registered in China for the treatment of non-small cell lung cancer [4].

So far, little is known about the expression of endostatin in well-differentiated thyroid cancer and its potential usefulness as a prognostic factor [5, 6]. To our knowledge, there are no data describing serum endostatin levels in patients with thyroid cancer. The estimation of serum endostatin levels is very simple and more useful for clinicians in contrast to the measurement of tissue expression which demands complicated immunohistochemical procedures.

Furthermore, there are no data regarding the influence of endogenous TSH stimulation on serum endostatin levels. Therefore, our aim is to study whether:

— serum endostatin concentrations differ between patients with metastatic and non-metastatic well-differentiated thyroid cancer;
— serum endostatin levels in patients with remission are similar to those in healthy subjects;
— endogenous TSH stimulation results in a change in serum endostatin concentration in patients with differentiated thyroid cancer.

Material and methods

Inclusion and exclusion criteria

The study protocol was approved by the Ethical Committee of the *Collegium Medicum* in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland. All the patients gave informed consent.

The study included 68 patients treated in the Department of Endocrinology and Diabetology of the Nicolaus Copernicus University in Bydgoszcz in the years 2003–2006.

The inclusion criteria for the study were as follows:

1. Well-differentiated thyroid cancer after total or near total thyroidectomy.
2. Radioactive iodine ablation.
3. No other known malignancies.
4. Normal platelet count.

The exclusion criteria were as follows:

1. Diabetes mellitus,
2. Heart failure,
3. Abnormal platelet count — because of its potential effect on serum endostatin concentration.

The study group underwent the standard follow-up procedure:

— diagnostic WBS, Tg, anti-Tg antibodies, ultrasonography (USG) of the neck after endogenous TSH stimulation (TSH > 30 uIU/ml), 7–8 months after 131-I treatment;
— frequency of the same procedure — in the case of full remission: in low risk patients after 2 years, then every 5 years, in high risk patients — every year. In patients with persistent/recurrent disease — 7–8 months after the additional treatment with radioiodine;
— systematic physical examination, TSH and Tg measurements every 6 months during TSH suppression (TSH <0,1 uIU/ml).

Remission was defined as:

1. Tg levels during TSH stimulation less than 2 ng/ml.
2. Absence of anti-Tg antibodies.
3. Negative WBS with 24-hour uptake less than 0.1%.
4. Normal results of standard imaging procedures: USG of the neck, X-ray of the chest, or any other (CT, NMR, PET, if needed).

The study included a control group of 25 healthy volunteers, comparable in age and sex with the study group.

The inclusion criteria for the control group:

1. Normal USG of the neck.
2. TSH, platelet count within normal range.
3. No evidence of chronic or acute disease.

Serum samples

Blood samples were obtained as a part of the routine clinical evaluations (TSH, Tg, anti-Tg antibodies, blood morphology). Thirty minutes after donation, they were centrifuged for 15 minutes at 2500 × g. The serum was removed immediately, and stored at < –80°C.

Serum endostatin measurements

Serum endostatin measurements were performed in batches using the Quantikine human endostatin sandwich enzyme immunoassay technique (R&D Systems,

Minneapolis, MN). All assays were performed in duplicate.

Serum Tg measurements

Serum Tg was measured with an IRMA assay (LIAISON, Dia Sorin, Italy).
Detection limits — < 0.2 ng/ml
Reference values assuming patients with an intact thyroid — 0.2–70 ng/ml
Serum anti-Tg antibodies measurements
Serum anti-Tg antibodies were measured with an IRMA assay (LIAISON, Dia Sorin, Italy).
Detection limits: < 5 IU/ml
Reference values: 5–100 IU/ml.
Serum TSH measurements

Serum TSH was measured with an ultrasensitive CMIA assay (ARCHITECT YOU 8200, ABBOTT). Detection limits: < 0.0025 uIU/ml Reference values: 0.35–4.94 uIU/ml.

Statistical analysis

Normally distributed data were presented as mean ± SD. Data not normally distributed were presented as median values. For normally distributed data, the Student t-test was used. Nonparametric tests were used when the data was not normally distributed. The groups were compared using U Mann-Whitney's test or ANOVA Kruskal-Wallis' test. Relationships among variables were sought using Spearman's correlation coefficient. Paired data were analysed using Wilcoxon's test. Statistical significance was assumed when the P value was less than or equal to 0.05.

Results

Baseline serum samples were obtained from 68 patients — 59 women and 9 men.

The mean age at the time of the study was 44. 9 ± ± 12.3 years, the mean duration of the disease was 53.3 ± ± 45.8 months. Paired samples (during suppression and after endogenous TSH stimulation) were available from 34 patients, according to the follow-up procedures.

Histological classification revealed 46 (68%) papillary thyroid cancers, 18 (26%) follicular thyroid cancers, and 4 (6%) oxyphilic thyroid cancers.

Within the group, 54 (79.4%) patients reached the remission criteria. The remaining 14 (20.6%) patients showed biochemical and morphological evidence of metastatic disease.

Serum endostatin levels were not dependent on age, sex, histological type of cancer, duration of the disease, number of radioiodine doses, TSH levels during suppression, or the total dose of 131-I used in the treatment.

Table I. *Serum endostatin concentrations in each study group*

Tabela I. *Stężenia endostatyny w badanych grupach*

Endostatin [ng/ml]	n	Median	Min.	Max.
Remission suppression	52	105.3	62.4	330.9
Locoregional metastases suppression	7	92.71	71.23	172.75
Distant metastases suppression	7	141.95	119.17	179.04
Healthy	25	88.1	44.4	116.6

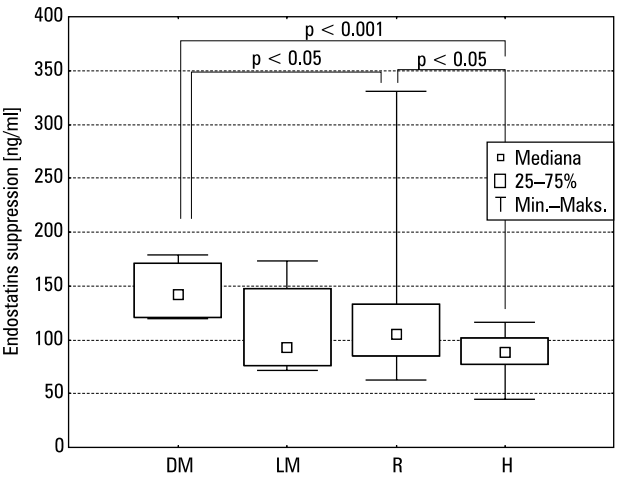

Figure 1. *Serum endostatin levels in patients with distant metastases (DM), locoregional metastases (LM), in remission (R), and healthy people (H)*

Rycina 1. *Stężenia endostatyny u pacjentów z przerzutami odległymi (DM), lokoregionalnymi (LM), w remisji (R) i u zdrowych osób (H)*

The concentrations of endostatin in each group are presented in Table I.

Serum endostatin concentration was significantly higher in patients with distant metastases of the thyroid cancer than in patients with locoregional ones, patients in remission, or healthy subjects (Fig. 1).

Serum endostatin concentrations in patients with locoregional metastases did not significantly differ from patients with remission and healthy people (Fig. 1).

The location of distant metastases and serum endostatin, Tg and TSH levels during suppression and stimulation are presented in Table II.

Patients with remission had higher endostatin concentrations than healthy people (Fig. 1).

During endogenous TSH stimulation provided in 34 patients with thyroid cancer, endostatin levels signi-

Table II. *Serum VEGF, Tg, and TSH levels during suppression and stimulation in patients with distant metastases*

Tabela II. *Stężenia VEGF, Tg i TSH w surowicy w czasie supresji i stymulacji u pacjentów z przerzutami odległymi*

TNM staging at the diagnosis	Location of metastases	Endostatin suppression [ng/ml]	Endostatin stimulation [ng/ml]	Tg suppression [ng/ml]	Tg stimulation [ng/ml]	TSH suppression [uIU/ml]	TSH stimulation [uIU/ml]
Follicular IV	Bones — > 20 foci	179.04	120.07	10.8	228.4	0.063	38.236
Papillary II	Non-iodine absorbing — mediastinum 3 foci, thyroid bed — 1 focus	138.45		13.6		0.015	
Papillary III	Lungs — 9 foci	120.45	91.93	Anti-Tg (+)	Anti-Tg (+)	0.013	68.300
Follicular II	Lungs — 4 foci, thyroid bed — 1 focus 4 cm	141.95		12.8	56.7	0.002	35.500
Papillary II	Lungs — 12 foci	142.22	95.26	4.9	16.8	0.025	44,800
Follicular IV	Non-iodine avid — multiple to hepar, bones, skin, left kidney	171.3		9615		0.025	
Papillary II	Multiple micrometastases to the lungs	142.22	95.26	4.88	16.8	0.03	

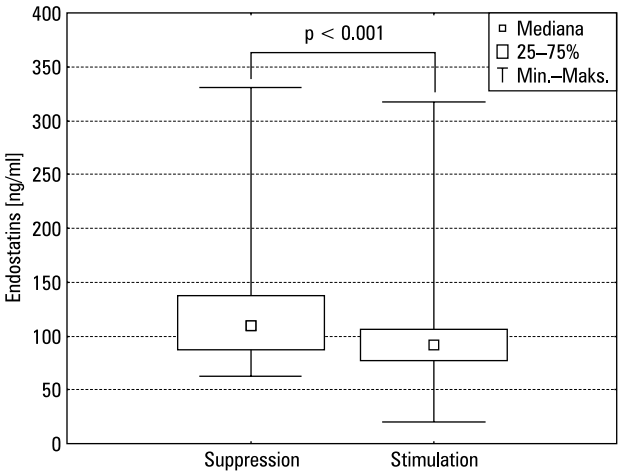

Figure 2. *Serum endostatin levels after TSH suppression and endogenous stimulation*

Rycina 2. *Stężenia endostatyny w trakcie supresji TSH oraz endogennej stymulacji TSH*

ficantly decreased (Fig. 2). This was observed in metastatic as well as non-metastatic patients.

Discussion

This study has demonstrated that patients with distant metastases have significantly higher median serum endostatin levels than patients with remission. However, patients with only locoregional metastases did not demonstrate increased serum endostatin levels compared with those with remission. Additionally, serum endostatin levels were higher in patients free of disease after the treatment than in healthy people. These observations suggest that endostatin plays a role in thyroid cancer biology.

Nevertheless, the clinical usefulness of endostatin as a marker of distant metastases is limited because there are overlapping data between the observed subgroups. Although endostatin levels in all patients with distant metastases were greater then median values in patients with remission and maximum values in healthy subjects, still the interpretation of high levels of endostatin is difficult. For example the highest value (330.9 ng/ml) was observed in a patient with no evidence of metastatic disease (Table I).

There is an interesting observation that patient 2 with non-iodine avid metastases, and patients 4, 5, and 7 (Table II) with relatively low Tg levels based on the extent of the disease, suggesting partial de-differentiation of their tumours, had very similar endostatin levels. Potentially, the use of endostatin as a disease marker in patients with tumours that do not efficiently make Tg would be useful, but requires further investigation.

To the best of our knowledge, there are no data describing serum endostatin levels in patients with well-differentiated thyroid cancer. There are several reports underlying the usefulness of measurements of serum

endostatin levels as a prognostic marker in gastric, hepatocellular carcinoma, renal cancer, non-Hodgin's lymphoma, and non-small cell lung cancer [7–11]. The higher serum endostatin levels in the studies presented above were correlated with advanced clinical stage of disease and poorer outcome.

There are very limited data relating to endostatin tissue expression in well-differentiated thyroid cancer. Hoffmann et al. demonstrated higher endostatin expression in primary tumor and metastatic lesions derived from human thyroid cancer compared to normal thyroid tissue, suggesting a possible association with tumor progression [5].

Given the antiangiogenic potential of endostatin, involved in a defence mechanism against further tumor growth, it is surprising that its high serum concentration is associated with metastatic disease.

There is evidence that antiangiogenic and antitumor activity of endostatin reveals a U-shaped curve with a specific optimal range responsible for higher activity [12, 13]. The theoretical explanation is that serum endostatin levels in patients with distant metastases, a more progressive disease, are not optimally active. The other possible explanation is that the dynamic balance between pro- and anti-angiogenic factors is still switched to proangiogenic activity. There are studies documenting the higher vascular endothelial growth factor (VEGF — the potent proangiogenic factor) levels in metastatic well-differentiated thyroid cancer [14–16]. The correlation between VEGF and endostatin levels was observed in different malignancies: renal cell carcinoma, colorectal cancer with liver metastases, non-Hodgin's lymphoma, and breast cancer [10, 17–19].

To the best of our knowledge, we report for the first time that endogenous TSH stimulation results in a decrease in serum endostatin levels. A possible theoretical explanation is that the most potent stimulus of well-differentiated thyroid cancer — TSH acts by decreasing the antiangiogenic defence mechanism. We observed significant decrease in serum endostatin levels in the whole study group, even in patients with remission — without the clinical and biochemical evidence of disease. These observations suggest that TSH might regulate endostatin synthesis through receptors located not only in follicular thyroid cells. The expression of TSH-receptors in tissues other than the thyroid gland was confirmed by Sorisky et al. [20].

The other possible explanation of the decrease in serum endostatin levels after TSH stimulation is the specific method of stimulation — the withdrawal of L-thyroxine. There is growing evidence that thyroid hormones are proangiogenic [21–24]. This effect is membrane-initiated at a hormone receptor site on the integrin $\alpha V\beta 3$ [25]. The decreased level of L-thyroxine (T_4) could lead to the inhibition of angiogenesis. There are data describing lower levels of angiogenesis stimulators, like VEGF, after T_4 withdrawal, so consequently, the lower endostatin levels observed in the present study may reflect the switch of the pro-/anti-angiogenic balance to the lower set-point [14, 26]. Another possible reason for the decrease in endostatin levels in whole study group is hypothyreosis after withdrawal of L-T_4 interpreted as an additional disease which might have influenced the angiogenesis. In fact, such lower endostatin levels in hypothyroidism have been previously described [27].

Conclusions

1. Median serum endostatin levels are higher in patients with distant metastases of thyroid cancer than in patients with locoregional metastases and patients in remission. The clinical usefulness of this finding is limited due to the overlapping data and the lack of a direct cut-off point indicating metastatic disease.
2. Endogenous TSH stimulation results in a decrease in serum endostatin levels in patients with or without thyroid cells, suggesting its regulatory effects through receptors located outside the thyrocytes.
3. Serum endostatin levels are not dependent on age, sex, histological type of cancer, duration of the disease, number of radioiodine doses, or the total dose of 131-I used in the treatment.

References

1. Folkman J. Antiangiogenesis in cancer therapy-endostatin and its mechanisms of action. Exp Cell Res 2006; 312: 594–607.
2. Abdollahi A, Hahnfeldt P, Maercker C et al. Endostatin's antiangiogenic signaling network. Mol Cell 2004; 13: 649–663.
3. Abdollahi A, Hlatky L, Huber PE. Endostatin: the logic of antiangiogenic therapy, Drug Resist Updat 2005; 8: 59–74.
4. Sun Y, Wang J, Liu Y et al. Results of phase III trial of rh-endostatin (YH-16) in advanced non-small cell lung cancer (NSCLC) patients. J Clin Oncol 2005; 23: 138 (Abstract).
5 Hoffmann S, Wunderlich A, Lingelbach S et al. Expression and secretion of endostatin in thyroid cancer. Ann Surg Oncol 2008; 15: 3601–3608.
6. Ye C, Feng C, Wang S et al. Antiangiogenic and antitumor effects of endostatin on follicular thyroid carcinoma. Endocrinology 2002; 143: 3522–3528.
7. Woo IS, Kim KA, Jeon HM et al. Pretreatment serum endostatin as a prognostic indicator in metastatic gastric carcinoma. Int J Cancer 2006; 119: 2901–2906.
8. Hu TH, Huang CC, Wu CL et al. Increased endostatin/collagen XVIII expression correlates with elevated VEGF level and poor prognosis in hepatocellular carcinoma. Mod Pathol 2005; 18: 663–672.
9. Feldman AL, Alexander HR Jr, Yang JC et al. Prospective analysis of circulating endostatin levels in patients with renal cell carcinoma. Cancer 2002; 95: 1637–1643.
10. Bono P, Teerenhovi L, Joensuu H. Elevated serum endostatin is associated with poor outcome in patients with non-Hodgkin lymphoma. Cancer 2003; 97: 2767–2775.
11. Iizasa T, Chang H, Suzuki M et al. Overexpression of collagen XVIII is associated with poor outcome and elevated levels of circulating serum endostatin in non-small cell lung cancer. Clin Cancer Res 2004; 10: 5361–5366.
12. Tjin Tham Sjin RM, Naspinski J et al. Endostatin therapy reveals a U-shaped curve for antitumor activity. Cancer Gene Ther 2006; 13: 619–627.

13. Celik I, Sürücü O, Dietz C et al. Therapeutic efficacy of endostatin exhibits a biphasic dose-response curve. Cancer Res 2005; 65: 11044–11050.
14. Klubo-Gwiezdzinska J, Junik R, Kopczynska E et al. The comparison of serum vascular endothelial growth factor levels between patients with metastatic and non-metastatic thyroid cancer, and patients with nontoxic multinodular goiter. Eur J Endocrinol 2007; 157: 521–527.
15. Tuttle RM, Fleisher M, Francis GL et al. Serum vascular endothelial growth factor levels are elevated in metastatic differentiated thyroid cancer but not increased by short-term TSH stimulation. J Clin Endocrinol Metab 2002; 87: 1737–1742.
16. Sorvillo F, Mazziotti G, Carbone A et al. Recombinant human thyrotropin reduces serum vascular endothelial growth factor levels in patients monitored for thyroid carcinoma even in the absence of thyroid tissue. J Clin Endocrinol Metab 2003; 88: 4818–4822.
17. Feldman AL, Alexander HR Jr, Bartlett DL et al. A prospective analysis of plasma endostatin levels in colorectal cancer patients with liver metastases. Ann Surg Oncol 2001; 8: 741–745.
18. Feldman AL, Tamarkin L, Paciotti GF et al. Serum endostatin levels are elevated and correlate with serum vascular endothelial growth factor levels in patients with stage IV clear cell renal cancer. Clin Cancer Res 2000; 6: 4628–4234.
19. Zhao J, Yan F, Ju H et al. Correlation between serum vascular endothelial growth factor and endostatin levels in patients with breast cancer. Cancer Lett. 2004; 204: 87–95.
20. Sorisky A, Bell A, Gagnon A. TSH receptor in adipose cells. Hormone and Metabolic Research 2000; 32: 468–474.
21. Davis FB, Mousa SA, O'Connor L et al. Proangiogenic action of thyroid hormone is fibroblast growth factor-dependent and is initiated at the cell surface. Circ Res 2004; 94: 1500–1506.
22. Mousa SA, Davis FB, Mohamed S et al. Pro-angiogenesis action of thyroid hormone and analogs in a three-dimensional in vitro microvascular endothelial sprouting model. Int Angiol 2006; 25: 407–413.
23. Reda M, Zickri M. Thyroxine as a stimulator of liver regeneration after partial hepatectomy:an experimental animal study. J Egypt Soc Parasitol 2008; 38: 853–861.
24. Jiang JY, Miyabayashi K, Nottola SA et al Thyroxine treatment stimulated ovarian follicular angiogenesis in immature hypothyroid rats. Histol Histopatho 2008; 23: 1387–1798.
25. Bergh JJ, Lin HY, Lansing L et al. Integrin alphaVbeta3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. Endocrinology 2005; 146: 2864–2871.
26. Dedecjus M, Kołomecki K, Brzeziński J et al. Influence of L-thyroxine administration on poor-platelet plasma VEGF concentrations in patients with induced short-term hypothyroidism, monitored for thyroid carcinoma. Endocr J 2007; 54: 63–69.
27. Kucharz EJ, Kotulska A, Kopeć M et al. Serum level of the circulating angiogenesis inhibitor endostatin in patients with hyperthyroidism or hypothyroidism. Wien Klin Wochenschr 115; 2003: 179–181.