

Changes and Prognostic Value of Serum Vascular Endothelial Growth Factor in Patients with Differentiated Thyroid Cancer

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Key Words

Vascular endothelial growth factor · Thyroglobulin · Thyroid · Cancer · Metastasis

Abstract

Objective: To evaluate the changes and the prognostic value of serum vascular endothelial growth factor (VEGF) in patients with differentiated thyroid cancer (DTC). **Subjects and Methods:** A total of 79 patients with DTC and 30 healthy individuals were divided into four groups: (1) a healthy control group (n = 30); (2) DTC without recurrence (n = 35; 23 papillary, 12 follicular); (3) DTC with local recurrence (n = 24; 15 papillary, 9 follicular), and (4) DTC with lung metastasis (n = 20; 13 papillary, 7 follicular). Serum VEGF and thyroglobulin levels were measured in all patients. **Results:** Serum levels of VEGF were significantly higher in the lung metastasis group than in the other three groups ($p < 0.05$). Serum thyroglobulin concentration positively correlated with VEGF expression ($r = 0.8678$, $p < 0.001$) in patients with thyroid cancer recurrence. Multivariate Cox regression analysis showed that clinical staging (OR = 1.851, 95% CI 1.04–3.47; $p = 0.038$), non-compliance with postoperative thyroxin replacement therapy (OR = 1.935, 95% CI 1.03–3.65; $p = 0.042$) and postoperative levels of thyroglobulin (OR = 1.892, 95% CI 1.01–3.56, $p =$

0.032) were independent predictors for thyroid cancer recurrence. Every additional 100 ng/l of serum VEGF levels increased the risk of thyroid cancer recurrence by 20.3%; but this did not reach statistical significance (OR = 1.203, 95% CI 0.95–1.52; $p = 0.125$). **Conclusions:** Serum VEGF increased in patients with recurrent thyroid cancer following surgical therapies. The predictive value of serum VEGF requires further investigation.

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Introduction

Differentiated thyroid cancer (DTC) is one of the most common head and neck cancers. Although many patients can survive after surgery and radioactive iodine treatment, some experience tumor recurrence or metastases [1]. Efforts have been made to develop biomarkers to predict cancer recurrence in order to facilitate early prevention or treatment of DTC. Several studies have demonstrated that there is a correlation between tumor growth or metastases and microvessel formation in DTC [2, 3]. Vascular endothelial growth factor (VEGF) is a glycoprotein that promotes endothelial regeneration, stimulates collateral blood vessel formation and increases vascular

permeability [4]. Recent studies have shown that tumor recurrence was related to tumor angiogenesis and VEGF was closely related to angiogenesis and growth invasion of thyroid papillary carcinoma [5]. High levels of serum VEGF and increased tissue expression of VEGF have been reported in patients with DTC [6–9]. Some studies have shown that the increase in serum VEGF correlates with local and distant metastasis in patients with thyroid cancer, and VEGF can be considered as an index of the presence of metastasis [5, 10–13]. However, another study showed that VEGF was decreased in patients with DTC [14]. Thyroglobulin measurement is primarily used to monitor DTC recurrence [15]. To date, there is limited data on the relationship between VEGF and thyroglobulin in patients with DTC, and the value of serum VEGF in predicting DTC recurrence following surgical therapy is uncertain. Therefore the aim of this study was to evaluate the changes in serum VEGF and their prognostic value for local and lung metastasis in patients with DTC.

Subjects and Methods

Patient Selection

The study was approved by the Ethics Committee of our hospital and informed written consent was obtained from all study participants. From March 2005 to September 2010, 79 consecutive patients with DTC were selected from the Department of Nuclear Medicine of our hospital. There were 53 women and 26 men, with a mean age of 39.5 ± 11.5 years (range, 16–68). Patients with the following conditions were excluded: (a) autoimmune disease; (b) coexistence of other cancers in addition to DTC; (c) pregnancy; (d) positive thyroglobulin antibody.

Physical exam, biopsy, and imaging tests (ultrasound, CT scan, MRI, chest X-ray, and nuclear medicine scans) were performed in each patient before surgery to assist with clinical staging of the DTC. The TNM staging system was used for preoperative staging. All patients were treated with thyroid resection, followed by radioiodine (^{131}I) treatment (80–120 mCi, median 100 mCi) in our department to destroy the postoperative residual thyroid tissues. In patients with local or distant metastasis, an additional course of ^{131}I treatment (120–150 mCi for local recurrence, and 150–200 mCi for distant recurrence) was administered 3 months after the first ^{131}I therapy. Thyroxin replacement therapy was prescribed to all patients following ^{131}I therapy.

In addition, a control group ($n = 30$) was recruited from the patients who presented for thyroid biopsy, whose thyroid was normal in tissue biopsy, thyroid ultrasonography and thyroid function tests. There were 19 women and 11 men, with a mean age of 40.8 ± 10.6 years (range, 19–65 years).

Specimen Collection and Testing

Fasting venous blood samples (5 ml) were collected in all patients after surgery and before ^{131}I therapy. Serum was separated and kept at -40°C in a refrigerator until analysis. Serum VEGF levels were measured by enzyme-linked immunosorbent assay

with a commercially available human VEGF kit (Hailang Biotechnology Co., Ltd., China). First, serum samples and standards were incubated with murine anti-VEGF monoclonal antibodies for 2 h, before enzyme-linked anti-VEGF polyclonal antibodies were added. Tetramethylbenzidine was used to display the intensity of the reactions. Optical density was measured at 450 nm using a VMax microplate reader and Softmax Pro software (Molecular Devices, Menlo Park, Calif., USA). All samples were run in duplicate, and a standard curve was established for each assay. Thyroid-stimulating hormone (TSH), thyroglobulin and thyroglobulin antibody concentrations were detected by chemiluminescence immunoassay methods (Beckman kits, USA). All samples of TSH and thyroglobulin were run in duplicates.

Follow-Ups

Neck ultrasound and chest CT studies were performed at 3, 6 and 12 months after ^{131}I treatment. Patients were divided into three groups: no recurrence, local recurrence and lung metastasis. Local recurrence was confirmed by fine-needle aspiration biopsy, and lung metastasis was confirmed by chest CT as well as ^{131}I imaging studies.

Statistics

Continuous data was expressed as mean \pm SD. Student's t test was used for comparison of data between groups. Spearman rank correlation analysis was used for the correlation study between serum VEGF and serum thyroglobulin concentrations. Cox regression analysis was applied for multivariate analysis of recurrence; $p < 0.05$ was considered as statistically significant. SPSS 10.0 statistical package (Chicago, Ill., USA) was used for statistical analysis.

Results

Follow-Up

In DTC patients, 51 had papillary carcinoma and 28 had follicular carcinoma. There was no statistically significant difference in age and sex between the DTC and control groups ($p > 0.05$). The follow-up time was between 12 and 72 months (mean 44.3 ± 4 months, median 47). A single course of ^{131}I treatment was applied to 23 patients. In the remaining patients with local or distant recurrence, 2, 3, 4 and 5 courses of ^{131}I therapy were applied to 12, 20, 18 and 6 patients, respectively. During follow-up, 4 patients had reoperation due to continued cervical lymph node enlargement. Two other patients also had cervical lymph node enlargement but were treated palliatively as the cervical lymph node and vascular adhesion were too extensive to be operated on. In addition, 2 patients died of pulmonary metastasis. Overall, 35 patients (23 papillary, 12 follicular) had no recurrence, 24 (15 of papillary, 9 of follicular) had local recurrence, and 20 (13 of papillary, 7 of follicular) had lung metastasis.

Table 1. Comparison of postoperative serum thyroglobulin and VEGF levels among groups

	Lung metastasis (n = 20)	Local recurrence (n = 24)	Without recurrence (n = 35)	Control (n = 30)	p
Age (median), years	38	41	34	43	>0.05
Male	4 (20%)	9 (37.5%)	13 (37.1%)	11 (36.7%)	>0.05
TSH, mIU/l	2.16 ± 0.80	2.03 ± 0.76	2.07 ± 0.68	2.12 ± 0.89	>0.05
Thyroglobulin, µg/l	77.52 ± 63.82*	35.35 ± 24.31*	1.27 ± 0.86		
VEGF, pg/ml	736.5 ± 248.2*	407.8 ± 57.6*	284.7 ± 48.8	268.6 ± 36.9	<0.05

* p < 0.01 compared with the nonrecurrence group and the control group.

Table 2. Comparison of postoperative serum VEGF in papillary and follicular thyroid cancer in the study groups

Groups	Papillary carcinoma (n)	Follicular carcinoma (n)	p
Lung metastasis	721.9 ± 308.5 (13)	756.0 ± 202.6 (7)	0.80
Local recurrence	400.8 ± 57.9 (15)	435.8 ± 57.8 (9)	0.16
Without recurrence	283.5 ± 51.7 (23)	289.9 ± 35.2 (12)	0.70

Table 3. Multivariate Cox regression analysis of predicting factors for thyroid cancer relapse

	OR (95% CI)	p
Sex	1.404 (0.63–3.13)	0.408
Age	0.996 (0.99–1.03)	0.822
Clinical staging	1.851 (1.04–3.47)	0.038
Pathological types	1.373 (0.96–2.48)	0.277
Noncompliance with thyroxin therapy	1.935 (1.03–3.65)	0.042
Thyroglobulin	1.892 (1.01–3.56)	0.032
VEGF	1.203 (0.95–1.52)	0.125

Comparison of Serum Thyroglobulin and VEGF Levels among Study Groups

The sensitivity of the VEGF assay was <7.0 pg/ml, with inter- and intra-assay variations of <5%, while the sensitivity of TSH and thyroglobulin assays was <0.1 mIU/l and 1.0 µg/l, respectively, with <5% inter- and intra-assay variations for both. In our laboratory, the reference range for TSH was 0.3–3.0 mIU/l, and for thyroglobulin it was 1.0–27.0 µg/l (mean 5.0 µg/l).

As shown in table 1, serum TSH levels were normal in all patients and there was no significant difference in serum TSH levels between the groups. The serum thyroglobulin concentration in the lung metastasis group was

higher than in the local recurrence and the nonrecurrence group (p < 0.01). The serum VEGF level in the lung metastasis group was higher than in the other three groups (p < 0.01). There was no statistically significant difference in serum VEGF levels between the nonrecurrent and the control group (p > 0.05). There was no statistically significant difference in serum VEGF levels in patients with different pathological types of DTC (table 2, p > 0.05). Correlation analysis showed that in patients with recurrent DTC, serum VEGF concentration positively correlated with thyroglobulin concentration (r = 0.8678, p < 0.001).

Factors of Thyroid Cancer Recurrence after Surgery

Six variables including gender, age, clinical stage (I, II or III), histological type (papillary or follicular), serum VEGF levels (every additional 100-ng/l assignment), and thyroglobulin levels have been taken into the multivariate Cox regression model. The multivariate Cox regression analysis showed that clinical staging (OR = 1.851, 95% CI 1.04–3.47; p = 0.038) and thyroglobulin levels (OR = 1.892, 95% CI 1.01–3.56, p = 0.032) were independent predictors for thyroid cancer recurrence (table 3). Noncompliance with postoperative thyroxin treatment was also an independent predictor for recurrence (OR = 1.935, 95% CI 1.03–3.65, p = 0.042). Every additional 100 ng/l of serum VEGF levels in-

creased the risk of thyroid cancer recurrence by 20.3%; but this did not reach statistical significance (OR = 1.203, 95% CI 0.95–1.52; $p = 0.125$, table 3).

Discussion

Our study showed that in patients with postoperative recurrence of DTC, there was an elevated serum level of VEGF. The level of VEGF in patients with lung metastasis was higher than in those with local recurrence. This study also showed that the serum level of VEGF in patients with recurrent DTC correlated with serum thyroglobulin levels. Furthermore, clinical staging, noncompliance with postoperative thyroxin therapy and serum thyroglobulin, but not serum VEGF, were independent predictors for the postoperative recurrence of DTC. These results suggest that although there was an increase in serum VEGF in patients with recurrent DTC, its value in predicting the prognosis of DTC requires further investigation.

It was reported that in patients with newly diagnosed thyroid cancer, serum VEGF levels were higher, but the postoperative serum VEGF level was reduced to normal [13]. However, previously reported serum VEGF concentrations in patients with thyroid cancer and the value of VEGF in predicting postoperative prognosis have been inconsistent [5–9, 10–14]. These inconsistencies may be related to the types of thyroid cancer, as different pathological types of thyroid cancer cells vary in their capacity to produce VEGF, and undifferentiated thyroid cancer and tumor cells produce more VEGF than other types [9–21]. The degree of differentiation of thyroid cancer was also related to the level of serum VEGF concentration [21, 22]. In the present study, however, the types of DTC (papillary or follicular) did not seem to have a significant impact on the serum VEGF levels, as the mean serum VEGF concentrations were similar between the two types of thyroid cancer (table 3). A significant difference in serum VEGF concentrations was found between patients

with recurrence or nonrecurrence, suggesting that the recurrence of carcinoma is a major determining factor of postoperative VEGF levels.

At present, the serum thyroglobulin concentration is considered the most sensitive means of recurrence monitoring in thyroid cancer [15]. However, the serum thyroglobulin concentration is often subject to interference of thyroglobulin antibodies, leading to false-negative occurrence. Our study showed that in patients with lung metastasis and local recurrence, both the serum thyroglobulin concentration and serum VEGF were increased, and there was a linear correlation between the two. Every additional 100 ng/l of serum VEGF levels increased the risk of thyroid cancer recurrence by 20.3%. However, multivariate regression analysis failed to show that VEGF was able to predict the recurrence of DTC. These results suggest that serum VEGF measurement may be used as a biomarker of thyroid cancer recurrence, but its interpretation must be in conjunction with other predictors such as clinical staging and serum thyroglobulin levels.

The most appropriate timing for blood sampling and VEGF assay in patients with DTC requires further investigation. It is possible that serum VEGF measured prior to thyroid surgery might have given better differentiation in outcome prediction. However, as the thoroughness of cancer resection and unsuspected residual disease also determine the outcomes of surgery, postoperative serum VEGF might be a better indicator of residual disease burden, and a better predictor of medium- to long-term prognosis.

Conclusions

In patients with recurrent DTC, serum VEGF was elevated and positively correlated with serum thyroglobulin. Serum VEGF may be used as a biomarker in addition to thyroglobulin for the assessment of the postoperative prognosis of DTC.

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