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Short Communication

A Study of Thyroglobulin Concentration in the Thyroid and Serum of Patients with Different Thyroid Disorders

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Abstract. Knowledge concerning the structure and quality of thyroglobulin (Tg) has great significance for the better understanding of the pathogenesis of different thyroid diseases. The localization of the *Tg* gene and studies of its structure by molecular biological techniques make possible precise investigations of its expression. The aim of our study was to evaluate Tg content in the thyroids and Tg concentrations in the serum of 108 patients suffering from benign or malignant thyroid disorders. The method of investigation was isolating total protein from thyroid tissues obtained during surgery and determining Tg content in the thyroid extracts and Tg concentrations in serum. The Tg concentrations in serum and in thyroid protein extracts were evaluated by fluoroimmunometric assay. Statistical analysis was carried out with the help of the computing programmes.

Key words: thyroglobulin; thyroid disorders; protein extracts.

Introduction

Thyroglobulin (Tg) is a glycoprotein which plays an important role in the synthesis and storage of thyroid hormones. This protein was isolated from thyroid tissues (mean 50–100 mg Tg/g tissue)[16]. The *Tg* gene covers approximately 300 kb pairs and is localized on the end of the long arm of chromosome 8q24[1, 17]. In somatic cell hybrids derived from a Burkitt lymphoma in which the oncogene c-myc was translocated from 8q24 to chromosome 14q32, the *Tg* gene was also translocated to chromosome 14[18]. Human *Tg* messenger RNA (mRNA) contains 8.5 kb and encodes at least 2700 amino acids[6, 11]. The relationship between Tg structure and function and the pathogenesis of thyroid diseases is a very important step in understanding how the disease can develop. Previous reports included a study of Tg in the thyroid tissue of patients with non-endemic and non-toxic nodular goiter[10], Graves-Basedow disease, congenital goiter, and benign adenomas[12–14, 19].

The aim of the present study was to evaluate Tg content and concentration in thyroid tissue as well as Tg serum concentration in thyroid disorders.

Materials and Methods

One hundred and eight patients with thyroid disorders were studied. There were 18 males and 90 females

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Table 1. General characteristic of 108 patients with thyroid disorders

Age	≤ 45 years	78
	> 45 years	30
Clinical features	solitary nodules	80
	multinodular goiter	10
	diffuse goiter	4
	not known	10
	palpable lymph node	4
Histologic characteristic		
simple goiter	colloid diffuse goiter	4
	colloid micro- and macrofollicularis	2
congenital goiter	colloid diffuse goiter	3
toxic nodular goiter	colloid nodules goiter	18
	colloid diffuse goiter	27
Graves-Basedow disease	parenchymatous toxic goiter	6
	colloid micro- and macrofollicularis	5
Metastatic lymph nodes from thyroid differentiated carcinoma		9
Hürthle adenoma	"Hürthle cell" adenoma	5
malignant tumors	papillary	22
	follicular	4
	anaplastic	3
Tg concentration in the tissue	normal 1.8–35 ng/ml	46
	high > 35 ng/ml	12
	low < 1.8 ng/ml	50
Tg content in the tissue	normal 50–100 mg/ml	38
	high > 100 mg/ml	60
	low < 50 ng/ml	10
Complementary treatment	^{131}I therapy	30
	thyroid hormone	108

with thyroid diseases in different stages of clinical evolution. The mean age of our patients was 46 years (range 12–69 years). The following cases were investigated:

- simple goiter (n = 6; 2.8%),
- congenital goiter due to a Tg synthesis defect (n = 3; 2.8%),
- toxic nodular goiter (n = 45; 41.6%),
- Graves-Basedow disease (n = 11; 10.2%),
- metastatic cervical lymph nodes from differentiated thyroid carcinoma (n = 9; 8.3%),
- Hürthle adenoma (n = 5; 4.6%),
- papillary thyroid carcinoma (n = 22; 20.5%),
- follicular thyroid carcinoma (n = 4; 3.7%),
- anaplastic thyroid carcinoma (n = 3; 2.7%).

All patients were treated surgically with one of the following options: total thyroidectomy (25 cases), subtotal thyroidectomy (76 cases) and cervical lymph node dissection (essentially non-radical homolateral, performed in 6 patients). Diagnosis of the disease was established on the basis of clinical, laboratory and histopathological signs. The clinical and histological data for these patients are shown in Table 1. The histological tumor classification was made according to the WHO criteria.

Bovine and rat thyroid tissue was used as a control.

Thyroid tissue specimens obtained at surgery were immediately frozen in liquid nitrogen and stored at –80°C. The methods of investigation were: isolation of total protein from the thyroid tissues obtained during surgery and the determination of Tg concentration in the thyroid extracts. Total protein was isolated by precipitation with ammonium sulphate and in accordance with the DIFFLEY and STILLMAN method[5] in the presence of PMSF as a proteinase inhibitor. The thyroid cells were macerated directly at 10°C by addition on an isolating solution containing 25 mM Tris HCl pH 7.5, 10 mM EDTA pH 8.0, 1 mM PMSF, and 20% glycerol alcohol.

After centrifugation at 10 000 × g for 20 min at 4°C, the pellets were desalinated in 4 M ammonium sulphate (final concentration 0.2 M). All pellets were incubated at 21°C and then centrifuged at 10 000 × g for 10 min. The protein supernatant was further analyzed. Protein concentrations were determined by the absorbance at 580 nm according to the BRADFORD method[3].

Tg concentrations in the thyroid protein extracts and in the serum were evaluated by fluoroimmunometric

assay (Delfia, Pharmacia, normal 50–100 mg/g; 1.7–35.0 ng/g).

Due to the lack of a normal distribution, statistical analysis was carried out with the help of the computing programmes Exel version 5.0 for Windows, and the Spearman Rank Order Correlation and the Mann-Whitney's tests. The observed differences were considered statistically significant if the probability of chance occurrence was $p<0.05$.

Results

The analysis of the isolated protein revealed a significant amount of Tg thyroid content (15.93±8.42 mg/g, normal 50–100 mg/g) and concentration (0.91± 0.48 ng/ml, normal 1.7–35.0 ng/g) in tissue and a high Tg serum concentration (103.45±9.66 ng/ml, normal 1.7–35.0 ng/g) in the metastatic nodes from thyroid differentiated carcinoma. In anaplastic cancers, the content of Tg was very low (1.63±0.48 mg/g) with concentrations of 0.96±0.97 ng/ml in tissue and 0.54±0.76 ng/ml in serum. The mean value of Tg content was 83.76±24.33 mg/g in tissue and 70.23±21.89 ng/ml in serum for the patients with follicular thyroid carcinoma. We also observed that in 3 instances of thyroid adenomas (Hürthle adenomas), the thyroid level was relatively high (Fig. 1, 2 and 3).

There were no significant differences in the levels of Tg in the thyroid among papillary cancer, Graves-Basedow disease, simple goiter and toxic nodular goiter. The mean Tg serum concentration was insignificantly higher in simple goiter (45.80±11.86 ng/ml) and Graves-Basedow disease (38.86±4.02 ng/ml); on the other hand, it was indeterminable in cases with congenital goiter due to a Tg synthesis defect (mean value 0.00±0.00 ng/ml; Fig. 3).

Thyroglobulin content in human thyroid in cases of simple goiter ranged from 81.55 to 245 mg/g (mean value 164.08±64.88 mg/g); in congenital goiter due to a synthesis defect its mean value was 722.85±39.98 mg/g of the tissue (Fig. 1, Table 2).

Using the Spearman Rank Order Correlation and the Mann-Whitney's test we determined the following correlations:

– a lack of correlation in thyroid Tg concentration vs. serum Tg concentration and in thyroid Tg content vs. serum Tg concentration in Hürthle adenomas and in papillary thyroid carcinomas,

– no statistical significance ($p<0.3$) in cases of simple goiter, congenital goiter due to a Tg synthesis defect and in metastatic lymph nodes from differentiated thyroid carcinomas,

– with the range of $0.3<p<0.5$, the correlation was on average in the Graves-Basedow disease and follicular thyroid carcinomas,

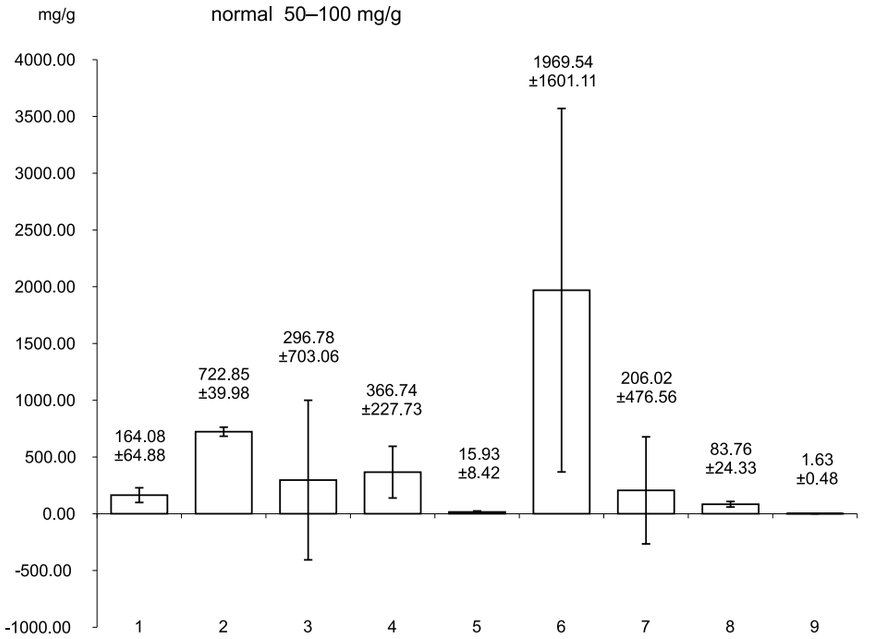

Fig. 1. Mean values of thyroglobulin content in thyroid tissues. 1 – simple goiter (n = 6), 2 – congenital goiter due to synthesis defect (n = 3), 3 – toxic nodular goiter (n = 45), 4 – Graves-Basedow disease (n = 11), 5 – metastatic lymph nodes from thyroid differentiated carcinoma (n = 9), 6 – Hürthle adenoma (n = 5), 7 – papillary thyroid carcinoma (n = 22), 8 – folliculary thyroid carcinoma (n = 4), 9 – anaplastic thyroid carcinoma (n = 3)

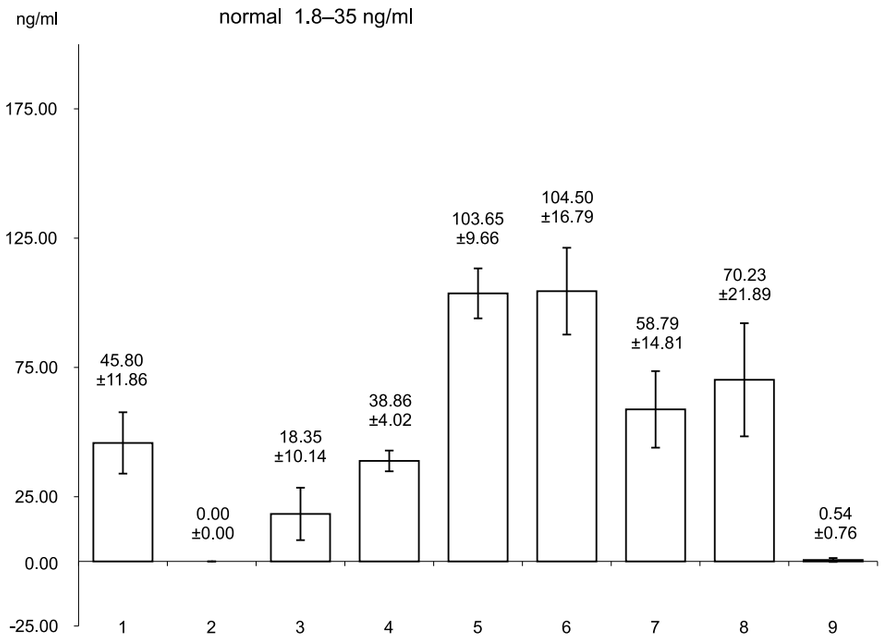

Fig. 2. Mean values of thyroglobulin concentration in serum. Explanations see Fig. 1

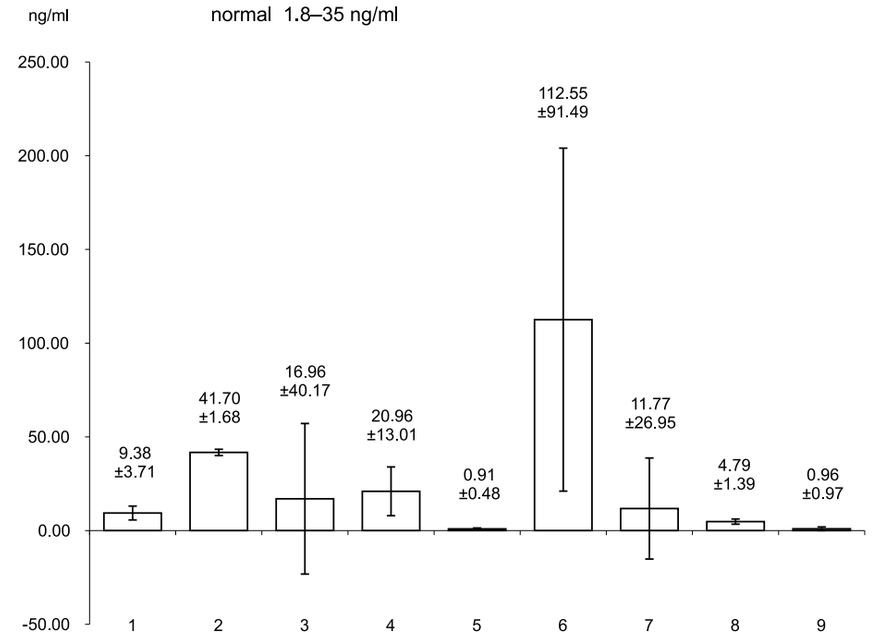

Fig. 3. Mean values of thyroglobulin concentration in thyroid tissues. Explanations see Fig. 1

– the correlation was statistically significant ($p<0.05$) in instances of toxic nodular goiter,

– in anaplastic thyroid carcinomas we determined a negative linear correlation ($p<0.5$) between thyroid Tg concentration and serum Tg concentration and a positive linear correlation ($p>0.5$) between thyroid Tg content and serum Tg concentration.

Discussion

Thyroglobulin is a recognized and uncommonly useful marker in thyroid neoplasm. This protein is an important prognostic factor indicating the presence of metastatic disease, and a rise in serum Tg in patients with known metastases indicates progression of the dis-

Table 2. Evaluation of thyroid Tg content and thyroid Tg concentration in studied cases

Clinical diagnosis	Thyroid Tg content [mg/g] (normal 50–100 mg/g)	Thyroid Tg concentration [ng/ml] (normal 1.8–35 ng/ml)
Simple goiter	164.08 ± 64.88	9.38 ± 3.71
Congenital goiter due to synthesis defect	728.88 ± 40.84	41.65 ± 2.33
Toxic nodular goiter	296.78 ± 703.06	16.96 ± 40.17
Graves-Basedow disease	366.74 ± 227.73	20.96 ± 13.01
Metastatic lymph nodes from thyroid differentiated carcinoma	15.93 ± 8.42	0.91 ± 0.48
Hürthle adenoma	1758.57 ± 1478.51	93.87 ± 77.57
Papillary thyroid carcinoma	206.02 ± 471.56	11.77 ± 26.95
Follicular thyroid carcinoma	83.76 ± 24.33	4.79 ± 1.39
Anaplastic thyroid carcinoma	1.40 ± 0.00	0.08 ± 0.00

ease. Since the first work of VAN HERLE et al.[16], several papers on Tg concentration have been published but only a few publications on the evaluation of Tg content in thyroid tissue[10, 12–14, 19].

We have demonstrated that the low levels of thyroid Tg content and concentration in differentiated carcinoma are a result of lower Tg expression and a rise of alternative splicing of a *Tg* gene transcript from its deletion fragments, as previously discribed[2, 4, 7, 12–14]. In cases of malignat neoplasms, Tg levels in thyroid tissue were undetectable. This was probably caused by the absence of the expression of mRNA Tg. We analyzed 5 cases of Hürthle adenoma and proved a high amount of Tg in the protein extracts. The patients in question had been treated with Methimazole before the operation because of hyperthyroidism, which might have stimulated the expression of the *Tg* gene.

We examined patients with simple goiter with normal serum TSH and Tg concentration where the goiter sizes were second degree according to WHO standards. Based on these criteria, we detected a normal range of thyroid Tg amount.

In 11 patients with Graves-Basedow disease, we observed an increase in tissue Tg content and concentration. This probably results from both cell and follicule structure disruption, or may also be connected with deglycosylation failure, possibly caused by a very strong induction of *Tg* gene expression. The increased synthesis of large amounts of Tg may be caused by an insufficient processing of the mature Tg protein[9]. This hypothesis, however, requires more detailed analysis of the Tg from the thyroid.

The Tg serum concentration was elevated in connection with various thyroid disorders, e.g. goiter, differentiated thyroid carcinoma and in methastatic lymph nodes from thyroid differentiated carcinomas. Assay of thyroglobulin in serum is of major importance for the follow-up of patients with thyroid carcinoma. Differentiated thyroid carcinomas of epithelial origin generally produce thyroid hormones and thyroglobulin. Follicular carcinomas usually cause higher serum levels than papillary carcinomas[8, 15]. Undifferentiated carcinomas produce thyroglobulin only occasionally. Differentiated carcinomas are mostly treated by surgery and ablation of residual thyroid tissue with radioactive iodine. After successful treatment, the levels suggest the presence of thyroid carcinoma if all normal thyroid tissue has been removed. If the thyroid carcinoma becomes undifferentiated, it may lose its ability to produce thyroglobulin.

In conclusion we found that: 1) the undetectable Tg levels in cases of malignat neoplasms in thyroid tissue and in the serum were probably caused by an expression defect of mRNA Tg; 2) no significant differences in the amount of Tg were noted in cases with the Graves' disease and toxic nodular goiter; and 3) increased synthesis of large amounts of Tg may be caused by insufficient processing of the mature Tg protein and have an influence on different thyroid disorders.

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