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The title of the article

The clinical utility of *Lens culinaris* Agglutinin-reactive thyroglobulin ratio in serum for distinguishing benign from malignant conditions of the thyroid

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Abstract

Background: Traditionally, the follow-up of differentiated thyroid carcinoma consists of periodic withdrawal from L-T4-suppressive therapy to allow performance of a highly sensitive serum Tg measurement to detect recurrences. In this study, *Lens culinaris* agglutinin-reactive thyroglobulin ratios in serum were investigated to evaluate in usefulness for detection of thyroid carcinoma.

Methods: The study was conducted on 93 serum sample from 23 healthy volunteers, 32 patients with benign thyroid tumor, 28 patients with thyroid carcinoma without

metastasis, and 10 patients with thyroid carcinoma with lymph node metastasis.
Results: The *Lens culinaris* agglutinin-reactive thyroglobulin ratio in patients with thyroid carcinoma was found to be significantly lower than that in patients with benign thyroid tumor in case with serum thyroglobulin concentration > 200 ng/ml. Among cases of thyroid carcinoma with lymph node metastasis, *Lens culinaris* agglutinin-reactive thyroglobulin ratios were significantly lower than in patient with thyroid carcinoma without metastasis and those with benign tumor regardless of serum thyroglobulin concentration.
Conclusion: Measurement of *Lens culinaris* agglutinin-reactive thyroglobulin ratio in serum may be useful for distinguishing between thyroid carcinoma and benign thyroid tumor.

1. Introduction

Thyroglobulin (Tg) is produced by the thyroid gland with a molecular weight of 660,000 and carbohydrate content of approximately 10%, and supports the synthesis of thyroxin and triiodothyronine [1-4]. The DNA and amino acid sequences of human Tg have been determined [5]. Serum Tg provides a specific and sensitive marker of disease activity in patients with differentiated thyroid carcinoma after total thyroidectomy [6]. Following total thyroidetomy detection of serum Tg suggests the presence of residual or recurrent disease. Any change in Tg levels reflects a change in tumor mass. In partial thyroidectomy or lobectomies circulating concentrations of Tg may reflect secretory capacity of both normal and malignant thyroid tissue [7-8]. At present, serum Tg is used for follow-up of thyroid carcinoma patients after treatment [9-11]. Traditionally, the follow-up of differentiated thyroid carcinoma consists of periodic withdrawal from L-T4-suppressive therapy to allow performance of a highly sensitive serum Tg measurement to detect recurrences. Also Pacini et al. showed that stimulated serum Tg is considered by most investigators to be the more sensitive and specific tool [12].

On the other hand, heterogeneity of the carbohydrate chains of Tg from thyroid carcinoma tissues has been reported [13-16]. Tg from thyroid carcinoma was not retain on Concanavalin A affinty columns, in contrast to Tg from normal thyroid tissue [13-14]. Concanavalin A could selectively retain > 95 % of serum Tg from patients with metastatic carcinoma, and the reactivity metastatic serum thyroglobulin and native thyroglobulin isolated from normal thyroid tissue toward

Concanavalin A were comparable [17]. The reactivity of Concanavalin A and *Ricinus communis* agglutinin-120 against Tg from papillary carcinoma was lower than that against Tg from normal, Graves' disease and benign tissues [18].

Lens culinaris agglutinin (LCA)-reactive α-fetoprotein (AFP) has been used as an effective marker for earlier diagnosis, assessment of therapeutic effect on and prognosis of hepatocellular carcinoma [19-22]. Measurement of LCA-reactive AFP has been carried out by competitive reaction between LCA and anti-AFP monoclonal antibody [23-24].

In our previous study, heterogeneity of the carbohydrate chains on Tg obtained from thyroid tissues was investigated with use of several lectins. Determination of *Lens culinaris* agglutinin (LCA) -reactive Tg ratio in tissues with use of competitive binding assay between LCA and anti-Tg monoclonal antibody was useful for distinguishing benign from malignant conditions of thyroid [25].

In this study, the LCA-reactive Tg ratio in serum was examined for usefulness in discrimination of thyroid carcinoma.

2. Materials and Methods

2.1 Clinical materials

Frozen sera were collected from 93 patients consisting of 23 healthy volunteers (12 males and 11 females), 32 patients with benign thyroid tumor (2 males and 30 females; 14 adenomatous goiters and 18 follicular adenomas), 28 patients with thyroid carcinoma without metastasis (5 males and 23 females; 27 papillary carcinoma, 1 anaplastic carcinoma), and 10 patients with thyroid carcinoma with lymph node metastasis (3 males and 7 females; all 10 papillary carcinoma). Benign tumor and thyroid carcinoma patients were diagnosed by pathologic examination. These sera were stored at -80 ℃. Informed consent was obtained from all patients.

2.2 LCA and anti-Tg monoclonal antibodies

LCA and two anti-Tg monoclonal antibodies (antibody clone numbers were WA-A and WA-C) having different epitopes were from Wako. Immunobeads (Immuno Chemical Inc., Okayama Japan) were coated with 80 μg/ml of anti-Tg antibody WA-A at 8 ℃ for overnight. Anti-Tg antibody WA-C was coupled with peroxidase (POD) as Fab'-POD using the conventional method.

2.3 Measurement of total Tg concentration

Fifty μl of serum sample and 100 μl of 50 mmol/l 3-morpholinopropanesulfonic acid buffer, pH 7.5 including 10 % Bloc Ace (Dainippon Pharmaceutical Co, Ltd., Osaka Japan) were mixed well, and one immunobead was added to the tube, and incubated at 8 ℃ for 40 min. The immunobead was washed three times in phosphate-buffered saline, buffer pH 7.5, and then incubated with 150 μl of 20 nmol/l Fab'-POD at 8 ℃ for 40 min. After repeating the wash step, 100 μl of 5 mmol/l luminescence substrate of 5-amino-2,3-dihydro-1,4-phtalazinedione sodium salt (Wako) and 100 μl of 0.02 % hydrogen peroxide were added and measured for luminescence on the AutoLumat Plus LB953 (Berthold Technologies GmBH & Co. KG., Bad Wildbad Germany). Tg concentration was determined using a calibration curve with Tg (HyTest Ltd., Turku, Finland).

2.4 Measurement of LCA-nonreactive Tg concentration

The serum sample and immunobead were incubated, washed, and measured for total Tg. The immunobead was then incubated with 150 μl of 1 mg/ml LCA at 8 ℃ overnight. After washing, the immunobead was incubated with Fab'-POD and luminescence was measured as described above.

2.5 Calculation of LCA-reactive Tg ratio

The LCA-reactive Tg ratio was calculated using the following equation:

LCA-reactive Tg ratio (%) = (Total Tg concentration – LCA-nonreactive Tg concentration) / Total Tg concentration × 100

2.6 Statistical methods

The significance of differences was examined using the Mann-Whitney *U* test and Welch's *t*-test.

3. Result

3.1 Assay precision and total Tg concentration and LCA-reactive Tg ratios in healthy volunteer, benign and malignant conditions.

The CVs of total Tg concentration and LCA-reactive Tg ratios were 3.7 - 7.1 % and 4.0

- 7.2 %, respectively.

Fig 1 shows total Tg concentration (A) and LCA-reactive Tg ratios (B), and significances of differences are shown in Table 1. In the benign thyroid tumor group, since neither total Tg concentration nor LCA-reactive Tg ratio differed significantly between adenomatous goiter and follicular adenoma, these conditions were grouped together as benign for comparison with malignant conditions.

In total Tg concentration, significant differences were found among the groups of healthy volunteers and patient with benign thyroid tumor or those with thyroid carcinoma without or with lymph node metastasis; however no significant differences were observed between benign and malignant conditions.

On the other hand, no significant difference between patients with benign tumor and those with thyroid carcinoma without metastasis was observed for LCA-reactive Tg ratio. However, the LCA-reactive Tg ratio for thyroid carcinoma with lymph node metastasis was significantly lower than those for benign tumor and thyroid carcinoma without metastasis.

3.2 LCA-reactive Tg ratio with respect to total Tg concentration

Patters of distribution of LCA-reactive Tg ratio with respect to total Tg concentration in serum are shown in Fig 2. All healthy volunteers had Tg concentration of < 50 ng/ml and all thyroid carcinoma with lymph node metastasis had Tg concentration more than 50 ng/ml.

For total Tg concentration less than 50ng/ml (Fig 2. A), LCA-reactive Tg ratio did not differ significantly among healthy volunteers, patients with benign tumors and those with thyroid carcinoma without metastasis.

For total Tg concentration of 51 to 200 ng/ml, LCA-reactive Tg ratio did not differ showing the significance between patients with benign tumors and those with thyroid carcinoma without metastasis, but significant differences in it were found between patients with thyroid carcinoma with lymph node metastasis and those with benign thyroid tumor or thyroid carcinoma without metastasis (Fig 2, B).

When total Tg concentration was > 200 ng/ml, LCA-reactive Tg ratio for patients with thyroid carcinoma without metastasis or with lymph node metastasis was significantly lower than that for benign thyroid tumor (Fig 2, C).

4. Discussion

It has been reported that total Tg concentration in serum cannot distinguish between benign and malignant thyroid diseases [26-28]. In our previous study, heterogeneity of the carbohydrate chains on Tg from thyroid tissues was investigated using LCA, and LCA-reactive Tg ratio for thyroid carcinoma tissues was significantly lower than those for normal, Graves' disease, and benign tumor tissues [25]. LCA-reactive AFP has been used as an aid of diagnosis of hepatocellular carcinoma, and has been was measured competitive assay between LCA and anti-AFP monoclonal antibody [23-24]. Since LCA-reactive Tg ratio may be a more specific marker for malignant thyroid conditions when serum Tg concentration is > 200 ng/ml, LCA-reactive Tg ratio in serum was investigated in patients with benign thyroid tumors and those with thyroid carcinoma with or without lymph node metastasis.

The LCA-reactive Tg ratio for patients with thyroid carcinoma with lymph node metastasis was found to be significantly lower than that for patients with thyroid carcinoma without metastasis. When serum Tg concentration was more than 200 ng/ml, LCA-reactive Tg ratio for thyroid carcinoma was significantly lower than that for benign thyroid tumor. LCA-reactive Tg ratio in serum may reflect malignant thyroid condition when Tg from thyroid carcinoma is dominant in serum. Measurement of LCA-reactive Tg ratio in serum may thus be useful for distinguishing of thyroid carcinoma from benign tumors.

Abbreviations:

Tg, thyroglobulin; LCA, *Lens culinaris* agglutinin; POD, peroxidase.

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Figure Legends

Fig 1

Total Tg concentration (A) and LCA-reactive Tg ratios (B) in healthy volunteers and thyroid diseases. The box and whiskers plots show the medians, 25th and 75th percentiles (box) and extreme low and high values (whiskers).

Fig 2

Distribution of LCA-reactive Tg ratios with respect to total Tg concentration:
(A) ≦ 50 ng/ml, (B) 51-200 ng/ml, (C) > 200 ng/ml. LCA-reactive Tg ratios are expressed as mean±S.D. NS, not significant (P-values > 0.01 were considered not significant). P-values were determined by Welch's t-test.

Fig 1

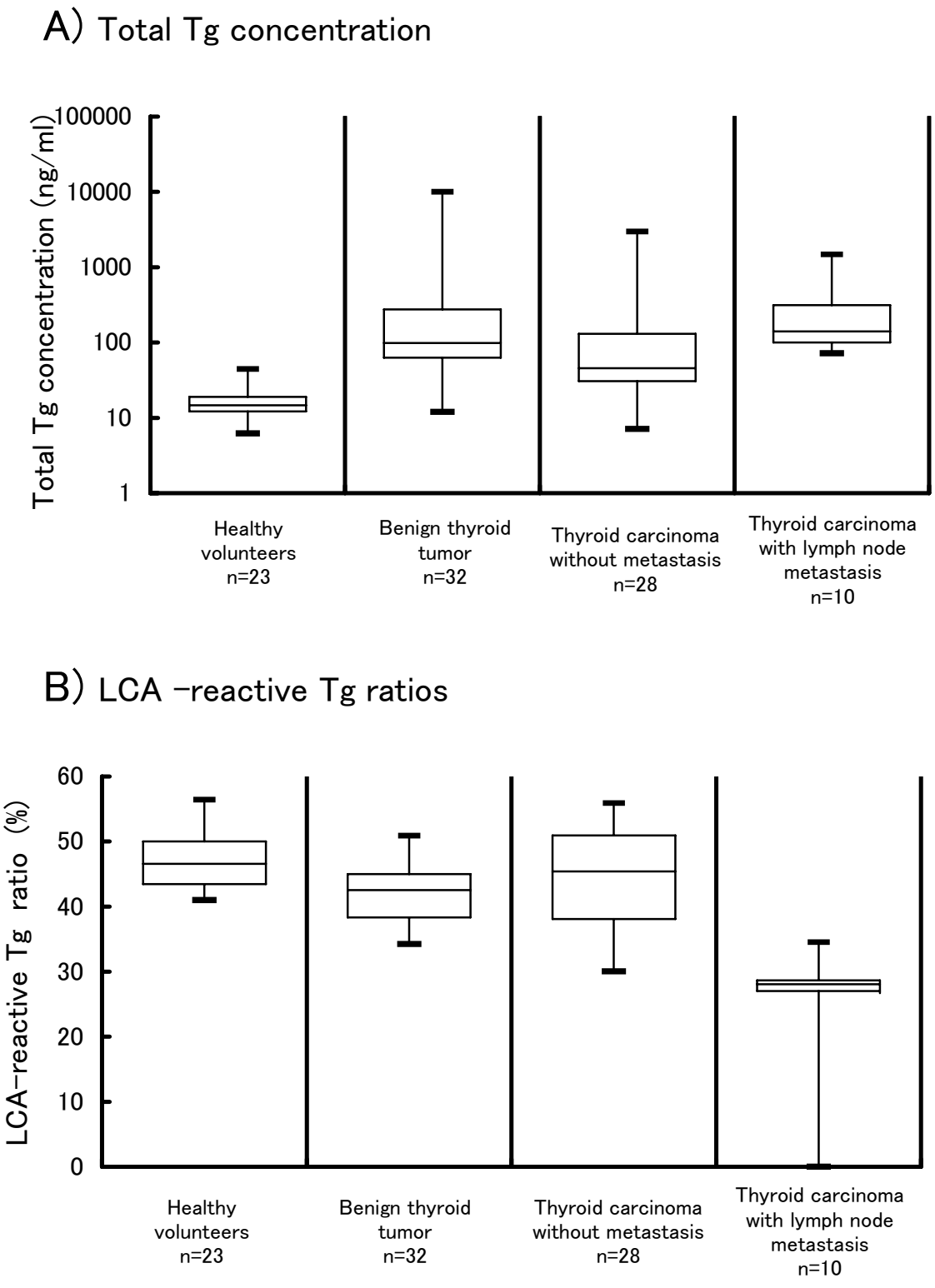
A) Total Tg concentration
Total Tg concentration (ng/ml)
100000
10000
1000
100
10
1
Healthy volunteers n=23
Benign thyroid tumor n=32
Thyroid carcinoma without metastasis n=28
Thyroid carcinoma with lymph node metastasis n=10
B) LCA −reactive Tg ratios
LCA−reactive Tg ratio (%)
60
50
40
30
20
10
0
Healthy volunteers n=23
Benign thyroid tumor n=32
Thyroid carcinoma without metastasis n=28
Thyroid carcinoma with lymph node metastasis n=10

Fig 2

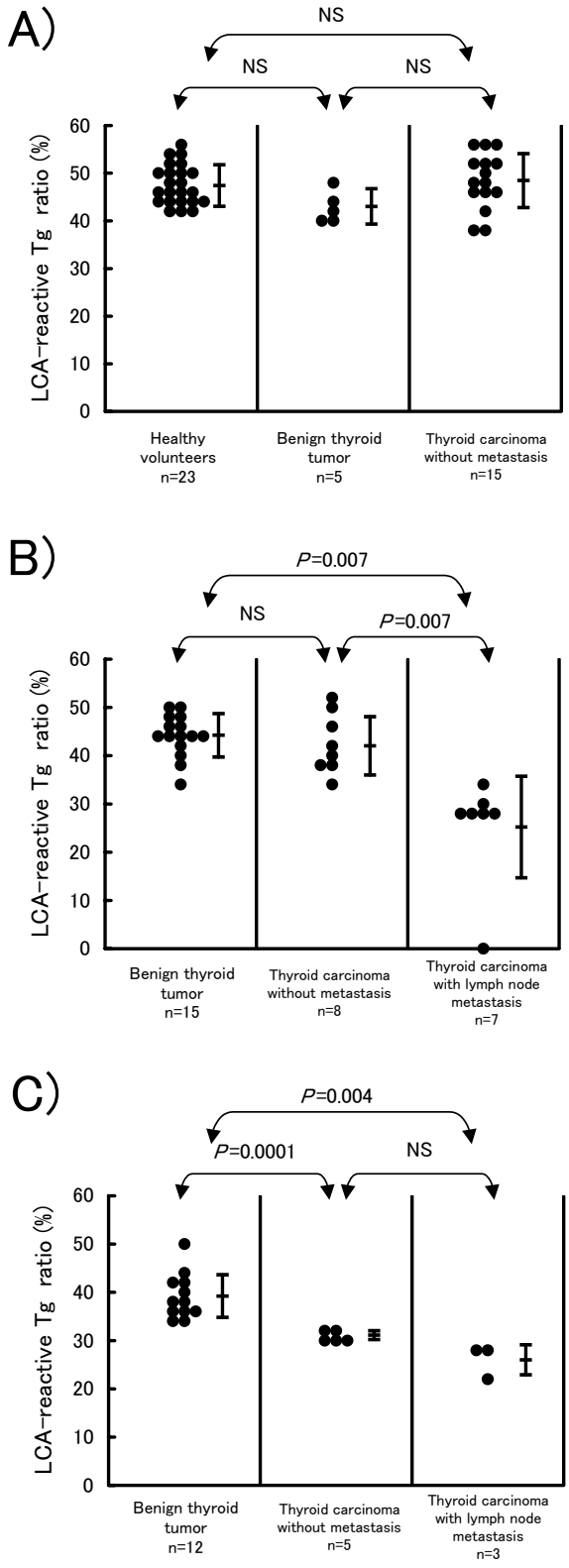
A)
NS
NS
NS
LCA-reactive Tg ratio (%)
60
50
40
30
20
10
0
Healthy volunteers n=23
Benign thyroid tumor n=5
Thyroid carcinoma without metastasis n=15
B)
P=0.007
NS
P=0.007
LCA-reactive Tg ratio (%)
60
50
40
30
20
10
0
Benign thyroid tumor n=15
Thyroid carcinoma without metastasis n=8
Thyroid carcinoma with lymph node metastasis n=7
C)
P=0.004
P=0.0001
NS
LCA-reactive Tg ratio (%)
60
50
40
30
20
10
0
Benign thyroid tumor n=12
Thyroid carcinoma without metastasis n=5
Thyroid carcinoma with lymph node metastasis n=3

Table 1

P–values for total Tg concentration and LCA–reactive Tg ratios in comparisons between patient groups

Patient group	*P*–values	
	Total Tg concentration[a]	LCA–reactive Tg ratios[b]
Healthy volunteers Benign thyroid tumor	<0.00001	0.0001
Healthy volunteers Thyroid carcinoma without metastasis	0.00001	NS
Healthy volunteers Thyroid carcinoma with lymph node metastasis	0.00001	<0.0001
Benign thyroid tumor Thyroid carcinoma without metastasis	NS	NS
Benign thyroid tumor Thyroid carcinoma with lymph node metastasis	NS	0.0004
Thyroid carcinoma without metastasis Thyroid carcinoma with lymph node metastasis	NS	0.0001

NS, not significant (*P*–values ≧ 0.01 were not considered significant.)

[a] *P*–values for Mann–Whitney *U*–test.

[b] *P*–values for Welch's *t*–test.