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NOTE

Urinary Iodine and Thyroid Antibodies in Okinawa, Yamagata, Hyogo, and Nagano, Japan: The Differences in Iodine Intake Do Not Affect Thyroid Antibody Positivity

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Abstract. Excess iodine intake may affect the development of Hashimoto's thyroiditis. Kelp consumption is very high in Okinawa. We expected a high prevalence of Hashimoto's thyroiditis in Okinawa. We studied urinary iodine excretion and the positivities of anti-thyroglobulin antibodies (TGAb) and anti-thyroid peroxidase antibodies (TPOAb) in the residents of Nishihara in Okinawa, Yamagata in Yamagata, Kobe in Hyogo, and Hotaka in Nagano, Japan. TGAb and/or TPOAb were positive in 142 (13.7%) of 1039 subjects in Nishihara, in 16 (16.0%) of 100 subjects in Yamagata, in 31 (13.4%) of 232 subjects in Kobe, and in 35 (13.9%) of 252 subjects in Hotaka; TGAb and/or TPOAb positivity was about the same in these 4 areas. One tenth of the subjects with positive TGAb and/or TPOAb had hypothyroidism; the frequencies of hypothyroidism in those with positive TGAb and/or TPOAb were about the same in Nishihara, Yamagata, Kobe, and Hotaka. The iodine concentration in samples of morning urine correlated well with the 24-h urine iodine excretion. The urinary iodine excretion was 1.5 mg/day in Nishihara. There were no differences between Nishihara and Yamagata in the urinary iodine concentration, but the urinary iodine concentrations in Kobe and Hotaka were less than those in Nishihara or Yamagata. The amounts of iodine excretion in Kobe and Hotaka were moderate, and less than those in Nishihara or Yamagata. The amounts of iodine intake in Kobe and Hotaka were less than those in Nishihara or Yamagata, but TGAb and/or TPOAb positivity was about the same in Nishihara, Yamagata, Kobe, and Hotaka. The differences in dietary iodine intake do not affect TGAb and/or TPOAb positivity.

Key words: Urinary iodine, Anti-TG antibody, Anti-TPO antibody

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AUTOIMMUNE thyroiditis, described by Hashimoto, is known as Hashimoto's thyroiditis [1]. Antithyroid antibodies were discovered in the sera of such patients [2]. Anti-thyroglobulin antibodies (TGAb) and/or anti-thyroid peroxidase antibodies (TPOAb) have been found in virtually all patients with Hashimoto's thyroiditis [3–5]. Positive TGAb and/or TPOAb in the sera is indicative of the presence of Hashimoto's thyroiditis [6–8].

Thyroid hormones, T4 and T3, contain iodine. An adequate supply of dietary iodine is essential for thyroid hormones synthesis, but excessive intake of iodine may induce thyroid dysfunction [9–14]. The Japanese are known to consume large amounts of iodine through seaweed, especially kelp (Laminaria). Kelp-induced endemic goiter has been

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reported to occur in the coastal regions of Hokkaido, the northern island of Japan [15]. Okinawa comprises dozens of southwestern islands in the Japanese archipelago. Kelp consumption, i.e. iodine intake, is thought to be very high in Okinawa. Evidence indicates that excess iodine has been reported to induce the destruction of thyroid cells and infiltration of lymphocytes in animals and in cultured thyroid cells, and affect the development of Hashimoto's thyroiditis [16–20]. Excess iodine intake has been reported to induce antithyroid antibodies; the increase in iodine in thyroglobulin molecules augmented the antigenecity of thyroglobulin [21, 22]. We therefore expected high TGAb and/or TPOAb positivity in Okinawa, but we did not have any data on iodine intake or TGAb and/or TPOAb positivity in Okinawa.

We intended to determine whether excess iodine intake may affect TGAb and/or TPOAb positivity or not. We studied TGAb and/or TPOAb positivity and urinary iodine excretion in the residents of Okinawa, and compared them with those in the residents of Yamagata (high kelp consumption area), and Hyogo and Nagano (moderate kelp consumption areas).

Subjects and Methods

Subjects and samples

TGAb and TPOAb were studied in 1039 subjects in Nishihara, Okinawa (age: 54.8 ± 14.1 years old (mean ± SD)), 100 subjects in Yamagata, Yamagata (53.5 ± 11.2 years old), 232 subjects in Kobe, Hyogo (52.3 ± 10.8 years old) and 252 subjects in Hotaka, Nagano (51.2 ± 13.2 years old). The iodine concentrations in morning spot urine specimens were measured in 150 of the 1039 subjects in Nishihara (65 males and 85 females; age, 51.9 ± 16.7 years old), 20 of the 100 subjects in Yamagata (10 males and 10 females, 51.0 ± 5.2 years old), 54 of the 232 subjects in Kobe (24 males and 30 females, 48.5 ± 6.3 years old), and 80 of the 252 subjects in Hotaka (40 males and 40 females, 49.5 ± 8.3 years old). We also correlated the iodine concentrations of morning spot urine specimens with those of urine collected over 24 h in 37 of the 150 Nishihara subjects. All subjects in this study had been stable residents of the respective areas for the previous 10 years. They were well nourished and healthy. Patients with Graves' disease and thyroxine-treated patients with hypothyroidism were excluded from the study. The subjects were not taking any drugs containing iodine, and had not been exposed to contrast media containing iodine.

Serum samples were obtained after overnight fasting and stored at −20 °C until assayed for thyroid antibodies, T3, T4 and TSH.

Analysis of urine iodine (UI) was done on 24-h urine specimens and morning spot urine specimens obtained after overnight fasting. Urine specimens were collected in shielded bottles containing 1 mL of 10% sodium azide, and stored in a refrigerator at 4 °C.

Measurements and statistical analysis

Anti-thyroglobulin antibodies (TGAb) and anti-TPO antibodies (TPOAb) were measured in sera by means of radioimmunoassay (RIA) kits (R.S.R. Limited, Cardiff, United Kingdom) [23]. A concentration greater than 0.4 U/mL was considered positive. The urine iodine (UI) was measured by an iodine selective electrode method [24, 25]; the UI values obtained by this electrode method and the chemical method were about equal [24, 25]. Serum concentrations of thyroxine (T4), triiodothyronine (T3) and TSH were determined by RIA with commercially available kits. The normal ranges for serum T4 and T3 were from 6 to 12 μg/dL (77–155 nmol/L) and from 78 to 182 ng/dL (1.2–2.8 nmol/L), respectively. The normal range for serum TSH was from 0.3 to 4.0 mU/L. All samples were tested in duplicate. Each value was expressed as the mean ± SD (standard deviation).

Statistical analysis was performed by the χ^2-test, Student's or Welch's t-test and the least squares regression analysis. P values less than 0.05 were considered to be statistically significant.

The study plan was reviewed and approved by our institutional review committee. Informed consent was obtained from all subjects.

Table 1. Anti-thyroglobulin antibody (TGAb) and anti-thyroid peroxidase antibody (TPOAb) in subjects living in Nishihara, Yamagata, Kobe, and Hotaka

Area (mean age ± SD)		No. of subjects	TGAb and/or TPOAb positive subjects (%)
Nishihara (54.8 ± 14.1)	Male+Female	1039	142 (13.7%)
	Male	321	19 (5.9%)]*
	Female	718	123 (17.1%)]*
Yamagata (53.5 ± 11.2)	Male+Female	100	16 (16.0%)
	Male	59	6 (10.2%)
	Female	41	10 (24.4%)
Kobe (52.3 ± 10.8)	Male+Female	232	31 (13.4%)
	Male	119	12 (10.1%)
	Female	113	19 (16.8%)
Hotaka (51.2 ± 13.2)	Male+Female	252	35 (13.9%)
	Male	125	10 (8.0%)]*
	Female	127	25 (19.7%)]*

* $P<0.01$.

Results

TGAb and TPOAb

We compared TGAb and/or TPOAb positivity in the Nishihara, Yamagata, Kobe, and Hotaka subjects (Table 1). The numbers of women with positive TGAb and/or TPOAb were greater than those of men in Nishihara and Hotaka (significantly different, $P<0.01$). The numbers of women with positive TGAb and/or TGAb in Yamagata and Kobe were greater than those of men (not significantly different). TGAb and/or TPOAb positivity was about the same in Nishihara, Yamagata, Kobe and Hotaka. The titers of positive TGAb and TPOAb were about the same in these four areas; the titers of TGAb and TPOAb were 5.6 ± 4.9 U/m*l* and 11.2 ± 7.5 U/m*l* in Nishihara, 4.7 ± 3.5 U/m*l* and 7.7 ± 4.3 U/m*l* in Yamagata, 4.1 ± 3.2 U/m*l* and 9.1 ± 6.5 U/m*l* in Kobe, and 5.1 ± 4.0 U/m*l* and 8.5 ± 6.1 U/m*l* in Hotaka. None of the TGAb negative and TPOAb negative subjects had hypothyroidism. Eight, 13, 10, and 9% of TGAb and/or TPOAb positive subjects in Nishihara, Yamagata, Kobe, and Hotaka, respectively, had hypothyroidism, and no differences in the basal TSH levels were noted among these patients with hypothyroidism in Nishihara, Yamagata, Kobe, and Hotaka.

Iodine concentrations in morning urine and 24-h collected urine

We studied the iodine concentrations in morning spot urine specimens in 150, 20, 54, and 80 residents of Nishihara, Yamagata, Kobe, and Hotaka, respectively. The urinary iodine concentrations of the subjects in Nishihara were not distributed normally, and ranged widely from 252 to 7580 μg/L (Fig. 1A). When these iodine concentrations were plotted on logarithmic scales, they were distributed normally (Fig. 1B). No differences in morning spot urine iodine concentrations were noted between males and females in Nishihara (male: 1.44 mg/L, female: 1.51 mg/L). Ranges of urinary iodine concentrations in Yamagata, Kobe, and Hotaka were from 973 to 3150 μg/L, from 413 to 2100 μg/L, and from 245 to 1900 μg/L, respectively. The iodine concentrations of morning spot urines in the subjects in Yamagata, Kobe, and Hotaka were also distributed normally on logarithmic scales.

We measured the iodine concentrations in morning spot urine samples and those in 24 h collected urine samples in 37 subjects. These 37 subjects were randomly chosen from the 150 Nishihara patients in Fig. 1. The mean urinary iodine excretion of the 37 subjects was 1.47 mg/day. Figure 2A demonstrates a positive correlation between morning urine iodine concentrations and

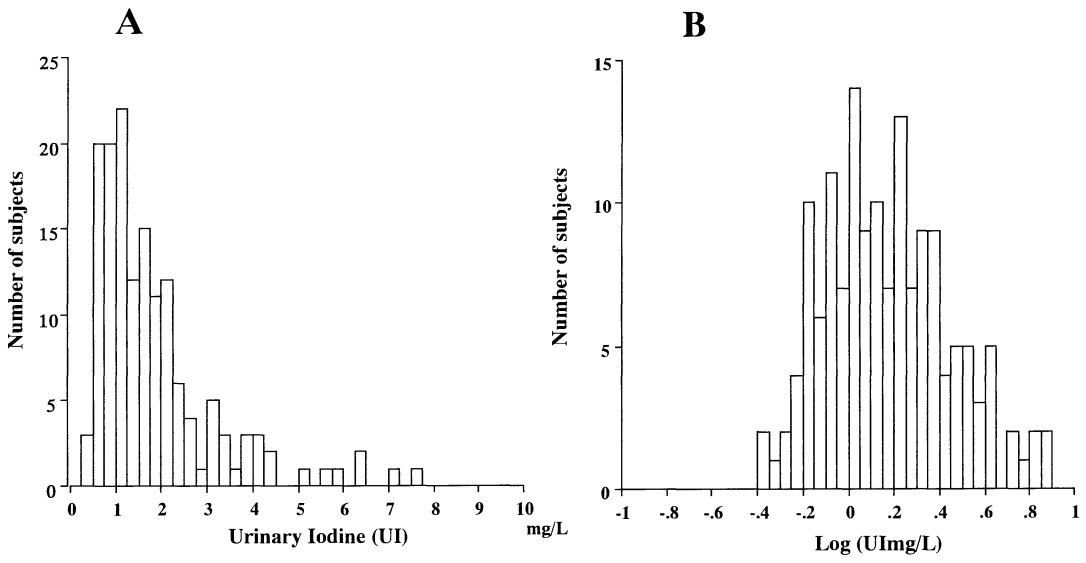

Fig. 1. A: Distribution of iodine concentrations in morning spot urine samples of 150 subjects (65 males and 85 females, age (mean ± SD); 51.9 ± 16.7 years) in Nishihara. Iodine concentrations in morning spot urine samples were not distributed normally. B: The iodine concentrations were distributed normally after logarithmic conversion. UI: urinary iodine.

those in 24-h collected urine specimens (r=0.86, y=0.51x + 0.41, $P<0.001$). Figure 2B demonstrates a positive correlation between morning spot urine iodine concentrations and iodine excretion for 24 h (r=0.71, y=0.68x + 0.83, $P<0.001$). These figures clearly show that the iodine concentrations in morning spot urine samples reflect those in 24-h collected urine specimens and 24 h iodine excretion.

Table 2 demonstrates the urinary iodine concentration in morning spot urine specimens. No differences were noted between the subjects in Nishihara and Yamagata, but the average urinary iodine concentrations in Kobe and Hotaka were less than those in Nishihara or Yamagata ($P<0.01$). Hotaka was the lowest urinary iodine concentration region among these four regions ($P<0.01$).

There was no correlation between the amounts of iodine excretion in urine and the TGAb and/or TPOAb positivity in these four regions.

Discussion

We studied TGAb and/or TPOAb positivity and urinary iodine excretion in the residents of Nishihara, Yamagata, Kobe, and Hotaka. The amounts of iodine excretion in Nishihara and Yamagata were larger than those in Kobe or Hotaka, but no differences in TGAb and/or TPOAb positivity were noted among Nishihara, Yamagata, Kobe, and Hotaka. The differences in iodine intake did not affect the TGAb and/or TPOAb positivity.

Moulopoulos *et al.* [26] reported that 200 to 500 μg of iodine was required to maintain a euthyroid state. Japanese are thought to ingest more than 300–500 μg of iodine daily. The quantity excreted in urine is only slightly less than the average daily dietary intake, reflecting scant loss of iodine through other avenues [5]. The subjects in Nishihara had 1.5 mg/day of urinary iodine excretion. This amount is higher than that reported in other parts of Japan; 470 μg/day (Nagoya) [27], 360 μg/day (Tokyo) [28] and 710 μg/day (Kyoto) [29]. The amounts of kelp consumed in Okinawa and Yamagata were reported to be high, but those in Hyogo and Nagano were reported to be moderate [30]. The urinary iodine concentrations in Kobe and Hotaka were less than those in Nishihara or Yamagata, suggesting that the amounts of iodine intake in the former were less than those in the latter.

TGAb and/or TPOAb positivity was about the same in Nishihara, Yamagata, Kobe, and Hotaka. In Nishihara and Hotaka, TGAb and/or TPOAb

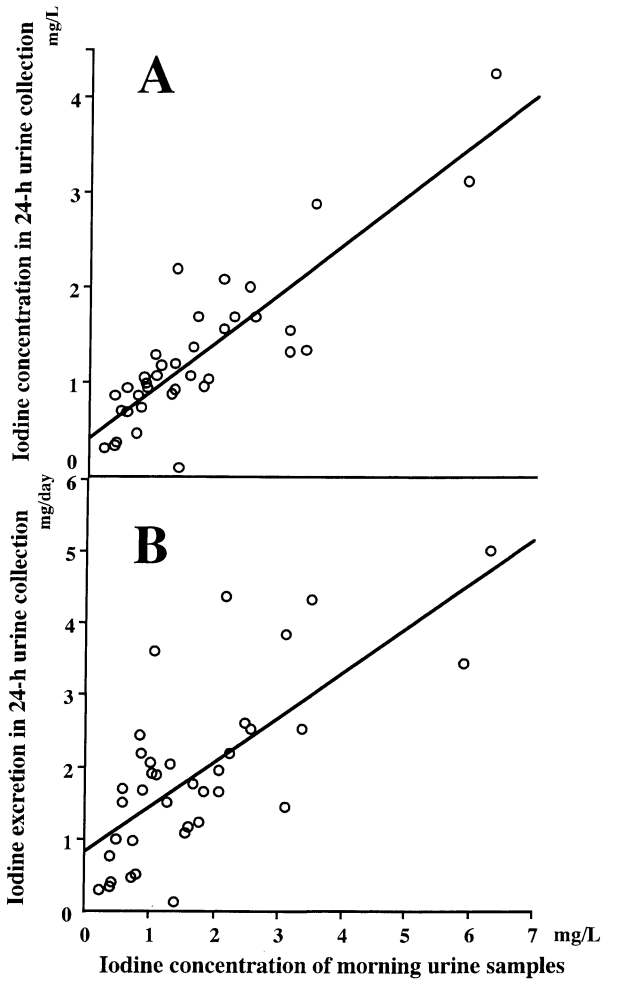

Fig. 2. A: Correlation of iodine concentrations in morning spot urine samples and 24-h collected urine samples in Nishihara. We studied 37 subjects (12 males and 25 females, age (mean ± SD), 59.2 ± 12.6 years). Iodine concentrations in morning spot urine and 24-h collected urine were positively correlated (r=0.86, y=0.51x + 0.41, $P<0.001$). B: Correlation of iodine concentrations in morning spot urine samples and iodine excretion in 24-h collected urine in Nishihara. Iodine concentrations in morning spot urine and 24-h urinary iodine excretion were positively correlated (r=0.71, y=0.68x + 0.83, $P<0.001$).

positivity of women was greater than that of men. None of the TGAb negative and TPOAb negative subjects had hypothyroidism, but one tenth of the

Table 2. Iodine concentration in morning spot urine

Area (No. of subjects)	Log (UI mg/L)	mean UI (mg/L)
Nishihara (n=150)	0.17 ± 0.28	1.48
Yamagata (n=20)	0.21 ± 0.16	1.62
Kobe (n=54)	0.08 ± 0.18	1.20
Hotaka (n=80)	−0.09 ± 0.16	0.81

NS, *, *, *, *

Values are the means ± SD. *$P<0.01$, NS, not significant; UI, urinary iodine.

TGAb and/or TPOAb positive subjects in Nishihara, Yamagata, Kobe, and Hotaka had hypothyroidism. These frequencies of hypothyroidism in TGAb and/or TPOAb positive subjects are the same as those reported elsewhere in Japan [31]. Subjects with positive TGAb and/or TPOAb do not always have hypothyroidism. Evidence indicates that excess iodine intake can affect the development of Hashimonto's thyroiditis [16–20]. Excess iodine intake has been reported to induce antithyroid antibodies [21, 22]. In humans, iodine enhances IgG synthesis of peripheral blood lymphocytes [32]. Radiologic contrast media and amiodarone, both containing large amounts of iodine, have been reported to play a role in the production of TGAb and TPOAb [33, 34]. People in Nishihara had high iodine excretion, and this high iodine excretion indicated that they had high iodine intake, but the frequency of TGAb and/or TPOAb positivity was about the same in all four localities studied. It seems that the differences in dietary iodine intake (from several hundreds to several thousands μg/day) do not affect the TGAb and/or TPOAb positivity in Japan. No significant differences were noted in TGAb and/or TPOAb positivity among several towns in England and Wales, although people in the different towns ingested different quantities of iodine [35]. The differences in iodine intake did not affect the TGAb and/or TPOAb positive in Japan. Dietary iodine may not affect TGAb and/or TPOAb positivity in Japan, although large doses of iodine was reported to play a role in thyroid-antibody production [33, 34].

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