Free Thyroxine Estimation for the Screening of Hyper- and Hypothyroidism in an Adult Population

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FUKAZAWA, H., SAKURADA, T., YOSHIDA, K., KITAOKA, H., NOMURA, T., SUZUKI, M., KAISE, N., KAISE, K., YAMAMOTO, M., SAITO, S. and YOSHINAGA, K. *Free Thyroxine Estimation for the Screening of Hyper- and Hypothyroidism in an Adult Population*. Tohoku J. exp. Med., 1986, **148** (4), 411-420 — Serum free thyroxine (FT₄), total T₄ (T₄) and total T₃ (T₃) were determined by radioimmunoassay in 1,114 adults during a periodic health evaluation to detect unsuspected thyroid dysfunction. As a result, 3 patients with hyperthyroidism, 4 with hypothyroidism, 3 taking thyroid medication, 4 with chronic thyroiditis, 1 with simple goiter and 3 women under estrogen administration were found. Free T₄ values were within the normal limits in the present four subjects taking estrogens, but all of them showed high T₄ and one high T₃ values. FT₄ and T₄ were low in four patients with hypothyroidism, but two of them showed normal T₃ values. For the screening of hyperthyroidism, FT₄ was the most reliable measurement and determination of either FT₄ or T₄ was suitable for the screening of hypothyroidism, but T₃ measurement did not diagnose all patients with hypothyroidism. ——— free thyroxine; thyroid dysfunction; mass-screening

Considerable evidence suggests that the concentration of serum free thyroxine (FT₄) is one of the best indices of the thyroid state. Recently, Remedios et al. (1980) proposed to add the FT₄I (Clark and Horm 1965; Hamada et al. 1970), the product of serum total T₄ and resin-sponge T₃-uptake, to the multiphasic evaluation for the detection of unsuspected thyroid dysfunction. On the other hand, radioimmunoassay (RIA) have been developed to estimate the serum FT₄ concentration in a routine basis.

The present paper describes the clinical significance of serum FT₄ determination by RIA for the screening of hyper- and hypothyroidism in an adult population during a periodic health evaluation.

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MATERIALS AND METHODS

Blood samples were obtained from 1,114 participants during a periodic health examination in 1980. They were inhabitants in two towns in Miyagi prefecture, Japan (239 males, 875 females). Their ages ranged from 20 to 69 years. Serum thyroid hormones were measured by radioimmunoassay with commercially available kits. The normal ranges of these parameters are as follows: serum FT₄ (GammaCoat two-steps FT₄ kit, Clinical Assay, Cambridge, Mass, USA.), 0.68 to 2.0 ng/100 ml; serum total T₄ (T₄, T₄-RIA kit, Clinical Assay), 4.5 to 11.5 µg/100 ml; and serum total T₃ (T₃, T₃-RIA-II kit, Dainabot Co., Japan), 87 to 185 ng/100 ml, respectively.

If thyroid dysfunction was suspected by this screening, the following examinations were undertaken: radioactive iodine thyroid scan and determinations of serum thyroid stimulating hormones (TSH, H-TSH kit, Daiichi RI Lab., Tokyo; normal, less than 8 µU/ml), serum thyroxine binding globulin (TBG) concentration (RIA-gnost TBG kit, Hoechst Co., Germany, normal range 13.6 to 31.6 µg/ml), resin-sponge T₃-uptake (T₃-RSU, Triosorb Test kit, Dainabot Co., normal range; 25 to 35%), 24-hr thyroid ¹³¹I-uptake (normal range; 10 to 40%), basal metabolic rate (BMR, normal range; -10~15%) and antithyroid antibodies. Anti-thyroid antibodies were determined with thyroglobulin and microsomal hemagglutination tests (MCHA and TGHA, Thyroid and Microsomal Tests, Fujizohki Co.). TGHA and MCHA were taken to be positive when titers are higher than 1:100.

In these subjects whose serum TSH values were higher than normal, thyrotropin-releasing hormone stimulating tests (TRH-test, 500 µg TRH, i.v.) were performed.

In each subject with abnormal thyroid hormone values, the clinical signs and symptoms were also evaluated.

The data were analyzed for significance using Student's unpaired *t* test, and *p* < 0.05 was considered to be significant.

RESULT

Serum FT₄, T₄ and T₃ concentrations in normal subjects

As shown in Fig. 1, FT₄ values of 1,096 euthyroid subjects showed a linear line on the logarithmic normal probability paper. Serum T₄ and T₃ of this group gave the same probability responses (data are not shown). Therefore, 95% limits of the normal ranges of serum FT₄, T₄ and T₃ were determined to be from 0.60 to 1.80 ng/100 ml, 6.0 to 11.8 µg/100 ml and from 84 to 176 ng/100 ml, respectively (Table 1).

Serum FT₄, T₄ and T₃ concentrations in the diagnosis of hyperthyroidism

Out of 1,114 participants, there were 26, 35 and 27 subjects who showed higher FT₄, T₄ and T₃ values than normal, respectively (Table 2A and 2B).

By the subsequent detailed examinations for thyroid function mentioned above, three patients who showed high T₃, T₄ and FT₄ values were diagnosed as hyperthyroidism (Table 3). These three patients showed no response to TRH test (data not shown). Hyperthyroidism is finally judged from the elevations of serum T₄, FT₄, T₃ and BMR values, increased ¹³¹I-uptake ratio and the absence of the TSH response after intravenous TRH administration.

Of the 26 subjects whose FT₄ values were higher than 1.81 ng/100 ml (Table

Fig. 1. Serum FT₄ values of normal subjects on logarithmic normal probability paper.

TABLE 1. Mean and 95% limits of serum FT₄, T₄ and T₃ in 1096 euthyroid subjects

	Total		Male	Female
	Mean $\pm$ S.D.	95% limits	Mean $\pm$ S.D.	Mean $\pm$ S.D.
FT ₄ (ng/100 ml)	1.05 $\pm$ 0.32	0.60 ~ 1.80	1.03 $\pm$ 0.30	1.06 $\pm$ 0.32
T ₄ (μ g/100 ml)	8.9 $\pm$ 1.5	6.0 ~ 11.8	8.8 $\pm$ 1.6	9.0 $\pm$ 1.5
T ₃ (ng/100 ml)	122 $\pm$ 33	84 ~ 176	125 $\pm$ 26	122 $\pm$ 35

2A), there was one patient with chronic thyroiditis taking thyroxine.

Of the 35 cases who had higher total T₄ than 11.9 μ g/100 ml, there were three women taking estrogens, including contraceptive pills, five patients with chronic thyroiditis and two with simple goiter. Two of them with chronic thyroiditis and one with simple goiter were taking thyroxine.

Of the 27 subjects having T₃ values higher than 177 ng/100 ml, one was taking estrogen and two were found to be treated with T₄ (one patient with chronic thyroiditis and the other with simple goiter).

The three subjects taking estrogens had normal FT₄ but T₄ higher than normal values. As for the T₃, one case showed high and the other two normal

TABLE 2A. *Distribution of serum FT₄, T₄ and T₃ levels in the 1,114 subjects*

FT ₄ (ng/100 ml)		T ₄ (μg/100 ml)		T ₃ (ng/100 ml)	
Cases (%)		Cases (%)		Cases (%)	
>1.81	26 (2.3)	> 11.9	4 (0.4)	>177	4 (0.4)
				176~84	0 (0)
				< 83	0 (0)
		11.8	22 (2.0)	>177	0 (0)
				176~84	22 (2.0)
				< 83	0 (0)
		< 5.9	0 (0)	>177	0 (0)
				176~84	0 (0)
				< 83	0 (0)
		1.80 { 0.60	1,060 (95.2)	> 11.9	31 (2.8)
176~84	24 (2.2)				
< 83	0 (0)				
11.8	1,005 (90.2)			>177	15 (1.3)
				176~84	970 (87.1)
				< 83	20 (1.8)
< 5.9	24 (2.2)			>177	1 (0.1)
				176~84	21 (1.9)
				< 83	2 (0.2)
<0.59	28 (2.5)			> 11.9	0 (0)
		176~84	0 (0)		
		< 83	0 (0)		
		11.8	21 (1.9)	>177	0 (0)
				176~84	21 (1.9)
				< 83	0 (0)
		< 5.9	7 (0.6)	>177	0 (0)
				176~84	5 (0.4)
				< 83	2 (0.2)

values.

Of the six patients with chronic thyroiditis, two cases were taking T₄ and had high T₄ values. In one of them, both FT₄ and T₃ were also high, but not in the other. In the remaining four patients with chronic thyroiditis taking no T₄, all FT₄ and T₃ were within the normal ranges, but T₄ was high in three and normal in one.

In two patients with simple goiter, the one taking T₄ showed high T₄ and T₃ values but normal FT₄, and the other with no T₄ administration showed high T₄

TABLE 2B. Comparison of diagnostic accuracy of serum FT₄, T₄ and T₃. "Estrogens" indicate patients taking pills or estrogenic medications

	Total number	FT ₄ (ng/100 ml)			T ₄ (μg/100 ml)			T ₃ (ng/100 ml)		
		1.81 ↑	1.80 ↓	0.59 ↓	11.9 ↑	11.8 ↓	5.9 ↓	177 ↑	176 ↓	83 ↓
Euthyroid	1,096	22	1,050	24	22	1,047	27	21	1,053	22
Hyperthyroid	3	3	0	0	3	0	0	3	0	0
Hypothyroid	4	0	0	4	0	0	4	0	2	2
Estrogens	3	0	3	0	3	0	0	1	2	0
Chr. thyroid.*										
T ₄ (+)	2	1	1	0	2	0	0	1	1	0
6 T ₄ (-)	4	0	4	0	3	1	0	0	4	0
Goiter										
T ₄ (+)	1	0	1	0	1	0	0	1	0	0
2 T ₄ (-)	1	0	1	0	1	0	0	0	1	0
Total Number	1,114	26	1,060	28	35	1,048	31	27	1,063	24

* Chronic thyroiditis.

TABLE 3. *Thyroidal function tests in 3 patients finally diagnosed as hyperthyroidism*

Patient No. (age, sex)	FT ₄ (ng/100 ml)	T ₄ (μg/100 ml)	T ₃ (ng/100 ml)	TSH (μU/ml)	T ₃ -RSU (%)	BMR (%)	24 hr ¹³¹ I-uptake (%)	Goiter
1 (55, M)	2.80	22.8	420	ND	42.5	+38.5	27.5	Unclear
2 (33, F)	4.90	24.0 ↑	393	ND	47.3	+70.0	65.4	Palpable
3 (46, F)	2.23	19.3	250	ND	33.4	—	33.7	Palpable
Normal range	0.60 1.80	6.0 11.8	84 176	<8	25 35	±10	10 40	

ND, not detectable.

TABLE 4. *Thyroidal function tests in 4 patients finally diagnosed as hypothyroidism*

Patient No. (age, sex)	FT ₄ (ng/100 ml)	T ₄ (μg/100 ml)	T ₃ (ng/100 ml)	TSH (μU/ml)	T ₃ -RSU (%)	BMR (%)	24 hr ¹³¹ I- uptake (%)	Anti-thyroidal antibodies	
								TGHA titer	MCHA titer
1 (51, F)	0.23	1.5	63	90	21.2	-36.2	12.3	Palpable	1 : 1,600
2 (49, F)	0.35	4.9	145	27	27.3	-18.5	42.6	Unclear	1 : 25,600
3 (58, F)	0.25	1.7	68	135	24.1	+11.4	6.4	Palpable	1 : 100
4 (61, F)	0.12	3.1	88	92	22.1	+14.5	6.4	Unclear	<1 : 100
Normal range	0.60 1.80	6.0 11.8	84 176	<8	25 35	±10	10 40	<1 : 100	<1 : 100

TABLE 5. Serum FT₄ concentrations in different age group of both sexes

Age	Males			Females		
	Mean (ng/100 ml)	S.D.	Number examined	Mean (ng/100 ml)	S.D.	Number examined
20~29	0.81	0.30	2	0.96	0.22	20
30~39	1.24	0.38	7	0.99	0.30	38
40~49	1.02	0.29	85	1.05	0.33	281
50~59	1.03	0.30	89	1.08	0.32	399
60~69	1.03	0.33	53	1.07	0.32	122
20~69	1.03	0.30	236	1.06	0.32	860

but normal FT₄ and T₃ values.

Consequently, there were ten cases with high T₄, three with high T₃ but only one with high FT₄ in women taking estrogen, patients with chronic thyroiditis and those with simple goiter.

Serum FT₄, T₄ and T₃ concentration in the diagnosis of hypothyroidism

Out of 1,114 adults screened, 28, 31 and 24 subjects showed lower FT₄, T₄ and T₃ values than normal, respectively (Table 2A and 2B).

Further detailed studies resulted in the diagnosis of four patients with hypothyroidism. All FT₄ and T₄ values were low in these four patients. T₃ levels were normal in two but low in the remaining two (Table 4). And these four patients showed the delayed hyperresponse to TRH tests (data not shown). The serum T₄, FT₄, T₃ and TSH, BMR and ¹³¹I-uptake values confirmed the diagnosis of primary hypothyroidism (Table 4).

In the other subjects who showed low FT₄, T₄ or T₃ value (Table 2A and 2B), T₃-RSU and TSH values were normal and antithyroid antibodies negative.

Serum FT₄ concentration in different age groups in both sexes

As shown in Table 5, 1,096 euthyroid males and females were grouped into five decades according to their ages (20-29, 30-39, 40-49, 50-59 and 60-69). No significant differences were found in serum FT₄ among any age-groups or between sexes.

DISCUSSION

The present study was undertaken to select the reliable thyroid function test out of T₄, T₃ and FT₄ determinations to detect the unsuspected patients with hyper- or hypothyroidism from numerous participants during periodic health examination. Because it is necessary to minimize the number of patients reexamined from the economical stand point of view. On the other hand, TSH-screening in current use for the detection of hypothyroidism seems to be not

suitable for the detection of hyperthyroidism, since there is no difference between TSH values in euthyroidism and in hyperthyroidism. Several RIA kits (Yoshida et al. 1980; Kitaoka 1981; Nakagawa et al. 1982; Suzuki et al. 1982) capable of measuring serum FT₄ have recently become available. We measured FT₄ by RIA for the screening of hyper- and hypothyroidism in 1,114 adults during a periodic health check up. The present study revealed that 1,096 subjects were euthyroid. Serum FT₄, T₄ and T₃ values in euthyroid subjects showed a distribution of logarithmic normal probability, similar to the results of the other serum chemical components such as uric acid and creatinine (Neuman 1968).

In the present study, free T₄ values were not changed with age between 40–69 years in agreement with similar data examining the FT₄ index (Bernudez et al. 1975; Gregerman and Davis 1978). Meanwhile, Malvaux et al. (1966) reported that FT₄ was decreased in adolescents as compared to adults. Although it was not significant, possibly owing to the limited number of subjects aged 20–49 years in the present study, FT₄ tended to be increased in 50's compared to 20's and 60's compared to 30's in females, and decreased in 40's compared to 30's in males.

Among 1,114 adults, there were 3 patients with hyperthyroidism (prevalence; 0.27%) and 4 with hypothyroidism (prevalence; 0.36%). When serum TSH levels were normal in the cases with low FT₄, T₄ and/or T₃, a TRH-test was not carried out in the present study. Therefore, there remains a possibility that they were in a state of subclinical hypothyroidism. Remedios et al. (1980) reported that there were 8 hyperthyroid (0.31%) and 14 hypothyroid (0.50%) patients in an apparently healthy population (2,704 adults) screened for unsuspected thyroid dysfunction using FT₄I determination. Baldwin and Rowett (1978) observed that the incidence was 0.39% for hypothyroidism. On the other hand, higher incidences, such as 0.97% and 1.58% (Baldwin and Rowett 1978; Tunbridge et al. 1977), have been reported for hyperthyroidism and 1.04% (Tunbridge et al. 1977) for hypothyroidism.

Recently extensive investigation has been made concerning the so-called "low T₃ syndrome" in nonthyroidal illnesses. This syndrome may be the result of diminution in extrathyroidal conversion of T₄ to T₃ (Chopra et al. 1975). In low T₃ syndrome, FT₄ has been reported to be normal despite a low FT₄I (Janni et al. 1981). In the present study, no case of apparent "low T₃ syndrome" was found, since we examined the subjects at a periodic health evaluation. However, it is conceivable that free T₄ measurements will be useful in the diagnosis of this syndrome, when low serum T₃ values are found during screening for thyroid dysfunction.

As shown in Table 2B, in the patients with chronic thyroiditis or simple goiter, seven patients showed high T₄, two high T₃ and one high FT₄ values with or without thyroxine administration. Total T₄ and T₃ values can be misleading if the patients have conditions that alter the concentration of thyroxine-binding proteins, such as pregnancy, estrogen administration (Utiger 1974), contraceptive

pills, and hereditary abnormalities in TBG (Sakurada et al. 1974). Free T₄ values were within the normal limits in the present four subjects taking estrogens, but all of them showed high T₄ and one high T₃ values. These results confirmed our previous observation (Yoshida et al. 1980) that the determination of FT₄ by GammaCoat FT₄ RIA kit was not influenced by the change in TBG.

It is generally accepted that FT₄I is a better index of thyroid status than total T₄ and total T₃ (Remedios et al. 1980). However, the FT₄I determination requires the measurement of both total T₄ and T₃-RSU. In addition, there is a question of the efficacy of FT₄I in patients with "low T₃ syndrome" (Chopra et al. 1979).

Out of 1,114 subjects examined, the number of cases reexamined for higher or lower than normal values were 54 (4.8%) with FT₄, 66 (5.9%) with T₄ and 51 (4.6%) with T₃. If the T₃ values is taken as the first choice for the screening, two patients with hypothyroidism as shown in Table 4 will be missed, although the number of cases for reexamination was least. Normal T₃ in patients with hypothyroidism is reported already in several papers (Lieblich and Utiger 1972; Chopra et al. 1973). The number of subjects for detailed reexamination was less when judged from FT₄ than from T₄.

In summary, determinations of serum FT₄, total T₄ and total T₃ were all sufficient methods for screening for hyperthyroidism. In the case of hypothyroidism, serum total T₃ measurement did not reveal all the patients with hypothyroidism.

We have shown that determination of FT₄ by RIA is useful as an initial screening test for thyroid dysfunction.

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