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Reference limits for serum thyrotropin in a Japanese population

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Abstract. The aim of the present study was to establish new reference intervals for serum thyrotropin (TSH) levels in Japanese subjects without antithyroid antibodies. We reviewed the serum TSH level of all patients 20 years of age and over who attended the outpatient clinic of our hospital between January 1, 2003, and September 20, 2010. The thyroid gland of every patient was examined by ultrasonography, and subjects found to have a normal thyroid were chosen. The following subjects were excluded: subjects with past history of thyroid diseases; subjects whose serum was positive for antithyroid antibodies; pregnant women; patients taking medication that might affect their free thyroxine (fT_4) level or TSH levels. Ultimately, 1388 subjects were included in the reference population. The serum TSH levels shifted to higher ranges as the age of the groups increased. The calculated reference range was 0.39-4.29 mIU/L in the 20-29-year-old group, 0.34-3.90 mIU/L in the 30-39-year-old group, 0.56-5.02 mIU/L in the 40-49-year-old group, 0.51-5.30 mIU/L in the 50-59-year-old group, 0.60-4.85 mIU/L in the 60-69-year-old group, 0.62-6.15 mIU/L in the over 70-year-old group. The results of this study showed that the upper limit of the normal range of serum TSH levels increased with age in a Japanese population. Since the number of elderly reference subjects was relatively small, further study is needed. Setting the age- and race-specific reference limits for serum TSH levels is important in order to prevent significant misclassifications of patients with abnormal TSH levels.

Key words: Thyrotropin, Reference limits, Antithyroid antibodies, Age, Ultrasonography

THE SERUM thyrotropin (TSH) level is the most sensitive index of thyroid function in apparently healthy subjects. The ongoing debate over subclinical hypothyroidism, its association with adverse health outcomes, recommendations for screening, and the use of levothyroxine for treatment has maintained the focus on defining the upper limit of the normal range of serum TSH levels.

Recent data from the United States have shown a progressive increase in TSH levels with age [1-3]. On the other hand, data from Germany, where the iodine supply is deficient, yielded lower TSH reference levels that declined with age [4-6].

In Japan, iodine intake is more than sufficient because of a diet that is rich in seaweed. Iodine intake may have an impact on the reference serum TSH concentrations of the population of a country. We reviewed the serum TSH levels of patients aged 20 years and over who had attended our outpatient clinic. All patients underwent thyroid ultrasonography and blood tests, including measurement of their serum thyroid hormone levels and testing for the presence of antithyroid antibodies. Patients found to have a normal thyroid gland were chosen as possible candidates for inclusion in the reference population of this study. Strict exclusion criteria were set. We then determined the distribution of the serum TSH levels and reference ranges of adult Japanese subjects who tested negative for the presence of serum antithyroid antibodies.

Patients and Methods

We reviewed the serum TSH levels of all 135,417 patients aged 20 years and over who had attended the outpatient clinic at Ito Hospital (Tokyo, Japan) between January 1, 2003, and September 30, 2010. Since our hospital is a specialized center for thyroid disorders,

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ultrasonography of the thyroid gland and blood tests that included measurement of serum thyroid hormone levels and testing for the presence of serum antithyroid antibodies were performed routinely. The definition of "normal thyroid gland" was thyroid with a homogenous echo pattern on thyroid images without any thyroid nodules, without goiter. The 5036 patients diagnosed as having a normal thyroid gland by ultrasonography were chosen as possible candidates for inclusion in the reference population of this study and their medical diagnosis was determined by reviewing their charts. Patients with past history of thyroid disorders were excluded. Subjects whose serum was positive for the presence of antithyroid antibodies (TSH receptor antibody, thyroid stimulating antibody, thyroglobulin antibody, TPOAb) were also excluded. We then excluded patients who were pregnant, who had been admitted to a hospital within the previous month, who were taking medication that might affect their free thyroxine (fT_4) level or TSH level (such as antithyroid drugs, thyroid hormones, iodine, lithium, amiodarone, estrogens, androgens, and steroid hormones and interferons), who had anemia, or a history of extrathyroidal malignancy, collagen disease, diabetes mellitus, neurologic disease, hepatic disorder, cardiovascular disease, or chronic kidney disease, or a familial history of autoimmune thyroiditis. Ultimately, 1388 of the patients were adopted as the reference population.

Laboratory methods

The TSH and fT_4 levels were measured by electrochemiluminescence immunoassays (ECLusys TSH and ECLusys fT_4, respectively; Roche Diagnostics GmbH, Mannheim, Germany). The manufacturer's reference limits were 0.2-4.5 mIU/L and 0.8-1.6 ng/dL, respectively.

Statistical analyses

The means, medians, standard deviation (SD), percentiles, P-values, and confidence intervals were calculated by using JMP software, version 8.0.2, (SAS Institute Inc., Cary, NC). Since the TSH data did not have a normal distribution, the frequency distribution curves for TSH were prepared conventionally by using log-transformed TSH values or Box-Cox transformed TSH values. We calculated the means ± 2SD of the TSH and fT_4 values. Turkey-Kramer's test was used to compare TSH values between different age groups. A *p*-value of <0.05 was considered significant.

Results

The 1388 subjects consisted of 1190 women and 198 men. The numbers of men and women according to age group were; 20-29 years (n=477, 424 women and 53 men); 30-39 years (n=440, 386 women and 54 men); 40-49 years (n=218, 183 women and 35 men); 50-59 years (n=132, 104 women and 28 men); 60-69 years (n=94 77 women and 17 men), and 70-80 years (n=27, 16 women and 11 men). We then calculated the mean ± 2SD of the serum TSH levels in each age group to compare them. Table 1 shows the means and reference intervals of the TSH levels according to age group and sex. The serum TSH levels of the entire study population ranged from 0.44-4.93 mIU/L, and this range was almost the same as the range of reference values (0.2-4.5 mIU/L) provided by the manufacturer of the immunoassay kit. We then investigated the distribution of TSH levels as a function of age, and, as shown in Table 1, the results showed that the TSH range shifted to higher values with age. Comparison between the 20-29-year-old group and the 70-80-year-old group showed a -2SD TSH level of 0.39 mIU/L and 0.63 mIU/L , respectively, a mean TSH level of 1.30 mIU/L and 1.96 mIU/L, respectively, and a +2SD level of was 4.29 mIU/L and 6.15 mIU/L, respectively. No sex differences in TSH distribution were observed in subjects up to 69 years of age, but the mean serum TSH level was significantly higher in the women in the 70 years of age and over group.

On the other hand, the distribution of serum fT_4 levels showed slightly lower in 40-49-year-old group compared to 20-29-year-old group (Table 2). In men, fT_4 levels were lower in the 60 years of age and over group compared to 20-29-year-old group. No statistically significant sex differences in serum fT_4 level were observed in any of the age groups.

Discussion

To the best of our knowledge this is the first study designed to establish reference ranges for serum TSH values in a Japanese population in which the results of an ultrasonographic examination of the thyroid gland and testing for the presence of antithyroid antibodies were performed in every subject. The results of this study showed that the upper limit of the serum TSH values of Japanese subjects whose serum tested negative for antithyroid antibodies increased with age.

Table 1 Means and reference intervals of serum thyrotropin (TSH) levels according to age group and sex

Age group	All patients (n=1388)				Women (n=1190)				Men (n=198)			
(years)		-2SD	mean	+2SD		-2SD	mean	+2SD		-2SD	mean	+2SD
All ages		0.44	1.48	4.93		0.45	1.48	4.82		0.39	1.48	5.66
	(n)				(n)				(n)			
20-29	477	0.39	1.30	4.29	424	0.38	1.28	4.30	53	0.48	1.42	4.15
30-39	440	0.34	1.53	3.90	386	0.46	1.44	4.46	54	0.24	1.54	4.43
40-49	218	0.56	1.67*	5.02	183	0.57	1.72*	5.25	35	0.54	1.43	3.82
50-59	132	0.51	1.65*	5.30	104	0.54	1.70*	5.31	28	0.42	1.48	5.22
60-69	94	0.60	2.10*	4.85	77	0.71	1.93*	5.25	17	0.22	2.68	5.14
>70	27	0.63	1.96*	6.15	16	0.83	2.39*	6.88	11	0.52	1.48	4.23

Serum TSH values (-2SD, mean, and +2SD) in mIU/L *p<0.05 for the difference from the 20-29-year-old age group

Table 2 Means and reference intervals of the serum free thyroxine (fT_4) levels according to age group and sex

Age group	All patients (n=1388)				Women (n=1190)				Men (n=198)			
(years)		-2SD	mean	+2SD		-2SD	mean	+2SD		-2SD	mean	+2SD
All ages		0.91	1.23	1.55		0.90	1.22	1.53		1.00	1.32	1.64
	(n)				(n)				(n)			
20-29	477	0.91	1.25	1.58	424	0.91	1.23	1.55	53	1.10	1.39	1.68
30-39	440	0.92	1.24	1.55	386	0.91	1.22	1.53	54	1.03	1.33	1.63
40-49	218	0.91	1.20*	1.50	183	0.90	1.19*	1.47	35	1.02	1.30	1.58
50-59	132	0.90	1.21	1.52	104	0.89	1.19	1.48	28	0.97	1.30	1.63
60-69	94	0.89	1.22	1.56	77	0.88	1.21	1.54	17	0.89	1.26*	1.63
>70	27	0.93	1.22	1.51	16	0.99	1.23	1.46	11	0.66	1.03*	1.40

Serum fT_4 values (-2SD, mean, and +2SD) in ng/dL *p<0.05 for the difference from the 20-29-year-old age group

Recent data from the United States, where the iodine supply is sufficient, has shown a progressive increase in the TSH levels with age [1]. They also showed that the median TSH value and reference limits were lower in African American and non-Hispanics, suggesting that the range of reference serum TSH values may vary according to race [1, 2, 7]. On the other hand, the TSH reference levels in an iodine deficient population were reported to be lower and to decrease with age [5]. Possible explanations for the shift to a higher TSH with aging include autoimmune changes related to long-standing residence in an iodine-replete environment[8], changes with age arising from a negative feedback relationship between fT_4 and TSH, and changes in TSH glycosylation [9]. A genetic study of an Ashkenazi Jewish population reported that the shift toward higher TSH concentrations and reference limits with age was associated with the presence of two single nucleotide polymorphisms (SNPs) in the regulatory/enhancer region of the TSH receptor gene[10]. The increased prevalence of these SNPs in other populations may provide a basis for the increase in TSH levels with aging. We did not investigate such characteristics of the subjects of the present study as body weight, body height, or smoking, because Kratsch reported that body weight, body height, body mass index, smoking, and time of blood collection did not affect TSH levels [4].

For the association between age and serum fT_4 levels, some study showed slight age-dependent decline in male in several studies, as shown in our study, no trend could be demonstrated in the female reference population [5, 11]. Other studies reported no differences in serum fT_4 concentration among age groups [7, 10, 12]. Sex hormones might have an impact on thyroid functions, and we excluded the subjects taking estrogens, androgens, and steroid hormones. The reason for the lower distribution of serum fT_4 levels in 40-49-year-old group in this study is unclear.

The establishment of an upper reference limit for serum TSH levels will have an impact on the controversy concerning the diagnosis and efficacy of treating mild subclinical hypothyroidism. Our study has limitation, because the number of elderly reference subjects, elderly male subjects, was relatively small. We set strict exclusion criteria to exclude factors that might affect thyroid status. Because the prevalence of ane-

mia and a history of extrathyroidal malignancy, diabetes mellitus, neurologic disease, hepatic disorder, cardiovascular disease, chronic kidney disease was higher among the elderly subjects than among the younger subjects, a larger proportion of the elderly candidates were excluded. The number of subjects over 70 years of age with normal thyroid but with extrathyroidal factors (such as anemia, a history of extrathyroidal malignaicies and so on) was 46 (28 women and 18 men). The calculated reference range for serum TSH levels was 0.64-8.24 mIU/L in all subjects, 0.7-8.95 mIU/L in women, and 0.56-7.15 mIU/L in men. The mean serum TSH level was not statistically different from the selected 27 subjects after exclusion of extrathyroidal factors over 70 years of age. Also, no difference was found in the serum fT_4 level between these two groups. The +2SD level of TSH was higher in 46 subjects with extrathyroidal factors, still, the number of subjects was small and thus the precision of the estimation was fairly low. Further study of a larger number of reference subjects is needed. Setting the age- and race-specific reference limits for serum TSH levels is important in order to avoid significant misclassifications of patients with abnormal TSH levels.

Disclosure Statement

The authors declare that they have no competing financial interests.

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