Measurement of Red Blood Cell Zinc Concentration with Zn-Test Kit: Discrimination between Hyperthyroid Graves' Disease and Transient Thyrotoxicosis

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Abstract. We have previously reported in patients with hyperthyroidism that the red blood cell (RBC) zinc (Zn) concentration reflects the mean thyroid hormone concentration over the preceding months. In the present study, the concentration of RBC Zn was measured by a simple and easy method with a Zn-test Wako kit. Within-run and between-run precision were 1.4% and 1.3%, respectively. The relationship between RBC concentration and dilution was linear. The average recovery was 103%. A good correlation ($r=0.97$) was obtained between this method and atomic absorption spectrophotometry. The mean concentration of RBC Zn in 39 euthyroid controls was 12.6 ± 1.3 mg/l, ranging from 10.4 to 15.1 mg/l. The RBC Zn concentrations in 38 patients with Graves' disease, in 10 patients with silent thyroiditis and in 3 patients with gestational thyrotoxicosis were 7.3 ± 1.6 (3.2-9.8), 12.0 ± 1.6 (9.5-14.2) and 11.8 ± 1.7 (10.5-13.7) mg/l, respectively. The concentration of RBC Zn was able to differentiate hyperthyroid Graves' disease from transient thyrotoxicosis except in 1 case and was a better index than TSH-binding inhibitory immunoglobulin. These results indicate that measuring RBC Zn with the Zinc-test Wako kit is very useful in differentiating hyperthyroid Graves' disease from transient thyrotoxicosis.

Key words: Zinc, Red blood cell, Graves' disease, Silent thyroiditis, Gestational thyrotoxicosis

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THERE are many reports indicating that the red blood cell (RBC) zinc (Zn) concentration is reduced in patients with hyperthyroidism [1-3], and is inversely related to the plasma T4 concentration [3]. We have previously reported [4-6] in patients with hyperthyroidism that the RBC Zn concentration reflects a patient's mean thyroid hormone level over the preceding months as glycosylated hemoglobin does in diabetic patients

[7]. Measurement of the RBC Zn concentration is therefore useful in differentiating hyperthyroid Graves' disease from transient thyrotoxicosis [5, 6]. But our previously reported method for measuring the RBC Zn concentration is troublesome [4]: packed RBC is block digested in the presence of acid (0.8 ml of concentrated nitric acid and 0.2 ml of concentrated perchloric acid in a micro Kjeldahl tube) and measured by atomic absorption spectrophotometry. In the present study, we describe a simple and easy method for measuring the RBC Zn concentration with a Zn-test Wako kit which is based on the method by Makino *et al.* [8].

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Table 1. Thyroid function tests in patients with transient thyrotoxicosis and comparisons with those in patients with Graves' disease

Transient thyrotoxicosis (case no.)	Age	Serum T4 (mg/dl)	Serum T3 (ng/dl)	RAIU (%)	TBII (%)
1. silent*	29	17.0	245	ND	7
2. silent	19	14.0	238	1.5	-9
3. silent	35	(2.2) [#]	(5.3) [#]	0.4	5
4. silent	32	(2.4) [#]	(6.2) [#]	0.3	-2
5. silent	37	20.0	276	0.7	8
6. silent	70	(4.4) [#]	(9.6) [#]	0.3	0
7. silent	31	10.9 (2.3) [#]	192 (5.4) [#]	ND	-2
8. silent	29	16.5	287	0.2	ND
9. silent	29	17.0	387	1.4	-2
10. silent	30	24.0	406	3.0	6
11. gestational**	29	19.7	220	ND	1
12. gestational	36	15.1 (2.6) [#]	142 (5.8) [#]	ND	0
13. gestational	26	24.0	220	ND	0
<u>mean ± SD</u>		<u>17.8 ± 4.2</u>	<u>261 ± 82^a</u>	<u>1.4 ± 1.9^a</u>	<u>0.8 ± 4.6^a</u>
Graves' disease (n=38)					
mean ± SD		20.3 ± 3.5	432 ± 170	60 ± 20	32 ± 23
range		14.0-24.0	272-800	27-90	-1-81
normal control (n=39)					
mean ± SD		7.3 ± 1.3	98 ± 13	ND	ND
range		5.3-10.0	73-114		
normal range		4.5-12.0	70-180	10-40	<10

silent*, silent thyroiditis; gestational**, gestational thyrotoxicosis; ()[#], serum free hormone concentration (free T4 ng/dl; free T3 pg/ml); ND, not determined. ^a: $P < 0.01$ vs. Graves' disease.

Materials and Methods

Heparinized venous blood was obtained from 39 healthy volunteers, 38 untreated patients with hyperthyroid Graves' disease, and 13 patients with transient thyrotoxicosis (10 with silent thyroiditis and 3 with gestational thyrotoxicosis) (Table 1). The diagnosis of Graves' disease was based on high serum T4 and T3 levels, a suppressed TSH concentration, diffuse goiter, and normal or high thyroid radioactive iodine uptake (RAIU) and/or positive TSH-binding inhibitory immunoglobulin (TBII). The diagnosis of silent thyroiditis was based on clinical and laboratory findings including a transient increase in serum T4 and T3 levels, a suppressed serum TSH concentration, and a marked depression of RAIU (patients Nos. 2-6, 8-10), or transient thyrotoxicosis followed by transient hypothyroidism during the postpartum nursing

period (patient Nos. 1, 7). None had pain in the thyroid gland. The diagnosis of gestational thyrotoxicosis was based on high T4 and T3 levels at 2-4 months of gestation, negative TBII, and spontaneous recovery in the second trimester. The hemoglobin (Hb) concentrations were all more than 10 g/dl.

RBC Zn was measured with a Zn-test Wako kit. Samples (packed RBC diluted with 10 volumes of distilled water, 0.5 ml) and trichloroacetic acid solution (0.5 ml) were pipetted into tubes and centrifuged for 10 min at 3000 rpm. Supernatants (0.5 ml) were transferred into each tube, and color reagents were added. Then the absorbance at 560 nm was measured within 30 min. The concentration of iron in the supernatants was less than 100 mg/dl. Iron concentrations less than 1 mg/dl had no effect on the Zn concentration in this kit. The RBC Zn concentration was also measured by atomic absorption spectrophotometry

[4, 5] in 13 patients to study the correlation with this method. Total serum T4 and T3 concentrations were measured by EIA; their normal ranges were 4.5–12 mg/dl and 70–180 ng/dl, respectively. Serum free T4 and free T3 concentrations were measured by RIA; their normal ranges were 0.7–1.8 ng/dl and 2.45–4.65 pg/ml, respectively. The serum TSH concentration was measured by a highly sensitive immunoradiometric assay. The normal range was 0.3–3.5 mU/ml. TBII was measured in 37 patients with Graves' disease and 12 patients with transient thyrotoxicosis with a TSH receptor assay kit (RSR Ltd., Pentwyn, Cardiff, UK). The normal range was less than 10%. The RAIU (normal range, 10–40%) was determined in 30 patients with Graves' disease and 8 patients with silent thyroiditis. It was not determined in patients with silent thyroiditis during breast-feeding or in patients with gestational thyrotoxicosis.

Statistical analyses were performed by means of Student's *t*-test. Correlations between two variables were calculated by linear regression analysis.

Results

Table 1 shows thyroid function tests in the patients with transient thyrotoxicosis compared with those in untreated Graves' disease and normal subjects. The mean serum T3 concentration was significantly ($P < 0.01$) higher in patients with Graves' disease than those in transient thyrotoxicosis, but there was a considerable overlap. There was no significant difference in the mean plasma T4 concentration. The plasma TSH concentrations were all less than the lower limit of sensitivity of 0.04 mU/ml in patients with Graves' disease and transient thyrotoxicosis.

TBII was negative in all 12 patients with transient thyrotoxicosis, but it was also negative in 16% (6/37) of Graves' disease. The RAIU value was less than 3% in patients with silent thyroiditis and more than 28% in patients with Graves' disease.

The concentration of RBC Zn measured with Zn-test Wako kits was correlated to a significant extent ($r=0.97$) with that measured by atomic absorption spectrophotometry (Fig. 1). The relationship between the RBC concentration and dilution was linear. The average recovery was found to be 103%. Within-run and between-run precision were 1.4%

Fig. 1. Correlation of RBC Zn concentrations measured with a Zn-test Wako kit and an atomic absorption spectrophotometer.

Fig. 2. RBC Zn concentrations in normal subjects and patients with thyroid diseases.

and 1.3%, respectively.

The RBC Zn levels in control subjects and in patients with transient thyrotoxicosis are shown in Fig. 2. The mean concentration of RBC Zn in 39 euthyroid controls was 12.6 ± 1.3 mg/l, ranging from 10.4 to 15.1 mg/l. The normal range defined as the mean ± 2 SD was 10.0–15.2 mg/l. The RBC Zn concentration was decreased in all 38 patients with Graves' disease. It was normal in 12/13

patients with transient thyrotoxicosis and one patient had a marginally low value (9.5 mg/l).

Discussion

Seino reported that the RBC Zn concentration in patients with iron deficiency anemia, whose hemoglobin concentration is more than 7.5 g/dl, is almost within normal limits when expressed per ml of packed RBCs, but it is lower per cell and is higher per gram of Hb, respectively [9]. Valberg *et al.* reported that the RBC Zn concentration in these patients is decreased per cell, but the level per volume and per gram of Hb is within normal limits [10]. In the present study we therefore expressed the RBC Zn concentration per liter of packed RBCs.

Silent thyroiditis accounts for 5–10% of all cases of thyrotoxicosis [11]. Although a thyroid RAIU value is most useful in differentiating hyperthyroid Graves' disease from silent thyroiditis, this test can be performed in only a limited number of institutions. TBII is positive in patients with Graves' disease in 80–90%, but it is also positive in patients with silent thyroiditis in about 15% [12]. Treatment with antithyroid drugs for silent thyroiditis is inappropriate.

Gestational thyrotoxicosis is common during the first trimester of pregnancy. Treatment with antithyroid drugs is needed in patients with hyperthyroid Graves' disease with pregnancy, since untreated hyperthyroidism is associated with increased fetal loss, with premature labor, and with low birth weight [13]. On the other hand, the value of antithyroid drugs in gestational thyrotoxicosis is not established, and their use should probably be reserved for the later stage of pregnancy. Therefore, differentiation between these two conditions is important. As mentioned above, TBII is positive in only 80–90% of Graves' disease. Thyroid stimulating activity is positive not only in Graves' disease, but also in gestational thyrotoxicosis [14]. In the present study, TBII was negative in all 12 patients with transient thyrotoxicosis tested, but TBII was negative in 16% (6/37) of Graves' disease patients. Therefore, in some cases it is difficult to differentiate Graves' disease from transient thyrotoxicosis due to the existence of silent thyroiditis and gestational thyrotoxicosis.

We recently reported that thyroid hormone inhibits the synthesis of carbonic anhydrase I (CAI) isoenzyme in human erythroleukemic YN-1 cells [15] and in the normal developing RBC (burst-forming unit erythroid-derived cells) [16] at the mRNA level [17]. Since most of the zinc in RBC is present as a complex with CAI [18], the RBC Zn concentration correspondingly decreases in patients with hyperthyroidism [1–3]. Since the life span of RBC is 120 days, we serially measured both RBC CAI and Zn concentrations in hyperthyroid patients before and after treatment with antithyroid drugs and reported that both RBC CAI [19] and Zn [4] concentrations reflect the mean thyroid hormone level over the preceding months. Because the changes in the RBC Zn concentration after changes in thyroid function take time to develop, at their initial presentation patients with acute thyrotoxicosis due to subacute thyroiditis and silent thyroiditis have a normal RBC Zn concentration [5, 6]. Lao *et al.* [20] reported that measuring the RBC Zn concentration allows discrimination between preexisting hyperthyroidism and gestational thyrotoxicosis. Recently Ozawa reported in a preliminary form that measurement of the RBC Zn concentration by atomic absorption spectrophotometry is very useful in differentiating hyperthyroid Graves' disease from transient thyrotoxicosis [21].

In the present study we measured the RBC Zn concentration with a Zn-test Wako kit. This kit was developed to measure the serum Zn concentration. Since the RBC Zn concentration is about 10 times more than that in serum, samples were diluted with 10 volumes of distilled water before the assay. The level of RBC Zn measured by this method was almost same as that measured by atomic absorption spectrophotometry. Furthermore, the dilution test, recovery test and within-run and between-run precision were all satisfactory. Because the value was not influenced by iron, we think that the RBC Zn concentration can be measured precisely by means of this kit. It has several advantages over the previous atomic absorption spectrophotometric method. Time consuming acid treatment is omitted and an atomic absorption spectrophotometer is unnecessary. It is not expensive (50 tests for 12,000 yen), is simple and easy to perform and the results are obtained within 2 h.

In the present study, The RBC Zn concentration was decreased in patients with hyperthyroid Graves' disease, as reported previously [4]. The RBC Zn concentration was in the normal range in all patients except one with transient thyrotoxicosis. The RBC Zn concentration may therefore be a better index than TBII to differentiate hyperthyroid Graves' disease from transient thyrotoxicosis. These results indicate that measuring RBC Zn with this

kit is clinically very useful.

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