NOTE

Erythrocyte zinc concentration as an indicator to distinguish painless thyroiditis-associated transient hypothyroidism from permanent hypothyroidism

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Abstract. Red blood cell (RBC) zinc (Zn) concentration reflects a patient's mean thyroid hormone level over the preceding several months. The aim of this study was to examine whether RBC Zn level can be used as an indicator to distinguish painless thyroiditis-associated transient hypothyroidism (TH) from permanent hypothyroidism (PH). RBC Zn level was measured in 30 untreated PH patients with Hashimoto's thyroiditis and 7 untreated TH patients with painless thyroiditis in whom preceding transient thyrotoxicosis had been confirmed. RBC Zn concentration was significantly lower in TH patients than that in PH patients. There was a positive correlation between RBC Zn and serum TSH, and the latter was clearly lower in TH patients than that in PH patients. However, RBC Zn level was again significantly lower in TH patients than PH patients despite of the comparable serum TSH levels in both groups when RBC Zn was evaluated in patients with serum TSH levels of less than 50 mU/L. Thus TH patients could be identified with RBC Zn measurement, allowing us avoidance of unnecessarily prolonged T4 administration to them.

Key words: Hypothyroidism, Zinc, Red blood cell, Painless thyroiditis

THERE ARE two clinical presentations of hypothyroidism, permanent hypothyroidism (PH) and transient hypothyroidism (TH). PH patients require a lifelong T4 supplementation. In contrast, TH spontaneously resolves within several months and therefore TH patients basically need no or short-term supplementation. This type of hypothyroidism is often associated with destructive thyroiditis such as subacute or painless thyroiditis [1], postpartum thyroid dysfunction [2], interferon therapy for chronic viral hepatitis [3], TSH-blocking antibodies [4] and exposure to excess iodide [5]. Accordingly, it is important to distinguish TH from PH before starting T4 supplementation.

However, there are few indicators that can be used for this purpose.

Thyroid hormone inhibits the synthesis of red blood cell (RBC) carbonic anhydrase I (CAI) [6] with which most of zinc (Zn) in RBC is associated [7], thus decreasing RBC Zn concentrations [8]. We have previously shown that patients with Graves' hyperthyroidism have a RBC Zn level clearly lower than that of patients with destructive thyroiditis [9,10]. Our data have therefore demonstrated that the RBC Zn concentration reflects a patient's mean thyroid hormone level over the preceding several months as glycosylated hemoglobin level does in diabetic patients [11]. In contrast, RBC Zn levels in hypothyroid patients have received less attention. In our previous studies [9], hypothyroid patients have a RBC Zn concentration similar to that of healthy controls. However, RBC Zn level was not measured individually in each form of hypothyroidism. Considering the thyroid hor-

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mone action on RBC Zn, there is a possibility that TH patients may have lower RBC Zn concentrations than PH patients since transient thyrotoxicosis precedes the onset of TH in a number of patients with destructive thyroiditis [12]. In the present study, we accordingly tested our hypothesis that RBC Zn is a useful indicator to distinguish painless thyroiditis-associated TH patients from PH patients.

Materials and Methods

Heparinized venous blood was obtained from 30 untreated PH patients (14 males and 16 females) and 7 untreated TH patients (all females) when they visited the thyroid clinic at Tohoku University Hospital or Ohta General Hospital for the evaluation of overt hypothyroidism (decreased serum T4 accompanied by elevated serum TSH) between January 2003 and December 2006. Mean ages ($\pm$ SD) were 61.4 ± 14.3 (range, 24 to 88) and 36.0 ± 11.5 (26 to 58) for PH and TH ($p < 0.05$), respectively. There were no pregnant women and patients with polycythemia or emphysema. The diagnosis of PH was based on the requirement of replacement doses of T4. All the PH patients have been on T4 supplementation because of serum TSH elevation over the normal range (0.3–3.5 mU/L) after taper of T4 doses. They all suffered from Hashimoto's thyroiditis as they had diffuse goiter and anti-thyroglobulin and/or anti-thyroid peroxidase antibodies. None had experienced signs and symptoms related with thyrotoxicosis prior to the onset of hypothyroidism. The diagnosis of TH was based on spontaneous recovery from hypothyroidism within 6 months after the onset of illness. Transient thyrotoxicosis with increased T4 and decreased TSH preceded the development of hypothyroidism in all the TH patients. They all did not have detectable levels of TSH receptor antibody, thus leading to a diagnosis of painless thyroiditis. None of them received antithyroid drugs, β -blockers, or iodide during the thyrotoxic phase. There were no patients who had been treated with amiodarone for cardiac arrhythmias. In the TH patients, T4 supplementation, if any, during hypothyroid phase had been stopped within 6 months after the onset of hypothyroidism, without relapse of hypothyroidism. Informed consent was obtained from all the patients and this study was approved by the institutional review board of Tohoku University School of Medicine.

RBC Zn was measured using a Zn-test Wako kit

(Wako Pure Chemicals, Osaka, Japan) as described previously [13]. Packed RBC lysed with 10 volumes of distilled water were mixed with trichloroacetic acid solution and centrifuged for 10 min at 3000 rpm. Color reagents were added to supernatants and the absorbance at 560 nm was measured within 30 min. The RBC Zn concentration in euthyroid controls was 10.0–15.2 mg/L and intra- and inter-assay variances were less than 2 % as shown previously [13]. Previous studies have revealed no changes in RBC Zn with increasing age and also no sex difference [9]. The hemoglobin concentrations were all more than 10 g/dL. Serum free T4 (FT4) and TSH levels were determined with commercial kits (Fujirebio Inc., Tokyo, Japan) and their normal ranges were 0.7–1.8 ng/dL and 0.3–3.5 mU/L, respectively. In some patients, serum total T4 (normal range, 4.5–12 μ g/dL) was measured instead of FT4 using a commercial kit (Dainabot Co., Tokyo, Japan).

Data are shown as the mean $\pm$ SD. Statistical analyses were performed with Student's *t*-test. Associations between RBC Zn and serum TSH were evaluated by Spearman's rank correlation test. Receiver operator characteristic (ROC) curve analysis was carried out for RBC Zn using TH *versus* PH as the outcome measure. A level of $p < 0.05$ was considered statistically significant.

Results

As shown in Fig. 1A, untreated PH patients had higher RBC Zn concentrations as compared with untreated TH patients. In the PH group, 13 of 30 patients exhibited RBC Zn levels above the normal range and the other had normal RBC Zn concentrations. In contrast, the RBC Zn level was normal in all but one patient (9.2 mg/L) in the TH group. We performed a ROC analysis in order to evaluate the predictive value of the RBC Zn for discrimination of TH from PH (Fig. 1B). The area under the curve was 0.98. Optimal sensitivity of 85.7% and specificity of 96.7% were seen at a cut-off level of 12 mg/L. As illustrated in Fig. 1C, serum TSH levels in the PH group were significantly higher than those in the TH group. In contrast, there was no significant difference in serum FT4 levels between the two groups (0.26 ± 0.19 for PH and 0.42 ± 0.12 for TH, $p > 0.05$). A significant correlation was found between the RBC Zn and serum TSH ($r = 0.49$, $p < 0.01$, Fig. 1D), but not between the former and se-

Fig. 1 A, Red blood cell (RBC) zinc (Zn) concentrations in patients with painless thyroiditis-associated transient hypothyroidism (TH; n=7) and those with permanent hypothyroidism (PH; n=30). The shaded area represents the normal ranges of RBC Zn (10-15.2 mg/L). B, A receiver operator characteristic (ROC) curve analysis for RBC Zn using TH versus PH as the outcome measure. C, Serum TSH levels in TH and PH patients. D, Correlation of RBC Zn concentrations with serum TSH levels ($r=0.49$, $p<0.01$). * $p<0.01$.

Fig. 2 RBC Zn concentrations in PH (n=3) and TH (n=4) patients with serum TSH levels of less than 50 mU/L. * $p<0.05$.

rum FT4 (data not shown). These results proposed the possibility that lower RBC Zn levels in the TH group was associated with lower serum TSH concentrations, in other words, RBC Zn concentrations depended on serum TSH regardless of the precedence of transient thyrotoxicosis. To test this, we accordingly evaluated the RBC Zn levels in patients with serum TSH levels of less than 50 mU/L (n=3 for PH and n=4 for TH). Whereas serum TSH concentrations were comparable in both groups (25.0 ± 6.3 for PH and 30.3 ± 9.1 for TH, $p>0.05$), RBC Zn levels were significantly lower in the TH group than the PH group (Fig. 2).

Discussion

In the present study, we clearly demonstrate that RBC Zn concentrations are lower in painless thyroiditis-associated TH than PH, suggesting that the measurement of RBC Zn may be useful to distinguish TH patients from PH patients and thus to avoid unnecessary T4 supplementation in the former cases. The ROC analysis revealed that the highest predictive value of RBC Zn was seen at a cut-off level of 12 mg/L, suggesting that hypothyroid patients with RBC Zn levels of less than 12 mg/L may suffer from reversible form of hypothyroidism. Whereas there was a positive correlation between RBC Zn and serum TSH, RBC Zn concentrations were significantly lower in painless thyroiditis-associated TH patients than PH patients in spite of comparable serum TSH levels when the patients with serum TSH levels of less than 50 mU/L were analyzed. Accordingly, RBC Zn concentrations may reflect the precedence of transient thyrotoxicosis rather than serum TSH levels, in other words, the severity of hypothyroidism. This is supported by the absence of significant correlation between RBC Zn and serum FT4. However, it remains unclear whether the preceding transient thyrotoxicosis in the TH groups is the sole cause for the difference in RBC Zn levels between the two groups.

Since TH often occurs in patients with painless thyroiditis, especially during the postpartum period [2, 14], it needs to be differentiated from PH before starting T4 supplementation. Painless thyroiditis exhibits transient thyrotoxicosis followed by TH in many cases [12]. However, thyrotoxicosis is usually mild and even asymptomatic in about 8% of patients [15], allowing that it escapes detection and thus only hypothyroidism is recognizable in such cases [12]. Considering the fact that RBC Zn concentration reflects a patient's mean thyroid hormone level over the preceding several months [9, 10], the measurement of RBC Zn may also be useful to identify TH patients with asymptomatic or unrecognized thyrotoxicosis in addition to those with confirmed thyrotoxicosis. Thus further studies are required to test such possibility by measuring RBC Zn levels in TH patients without apparent thyrotoxic phase.

Previous studies have demonstrated that elevated thyroidal radioactive iodine uptake (RAIU) [5, 16], goiter [16, 17], serum TSH [17], TSH-dependent elevation of serum thyroglobulin (Tg) [18] and thy-

roid echogenicity [19] may predict the reversibility of hypothyroidism. However, the presence of goiter, RAIU and serum Tg level, which may reflect the number of preserved thyroid follicles, are less useful in Hashimoto's thyroiditis as compared with iodide-induced hypothyroidism [20]. In addition, RAIU and thyroid ultrasonography are time consuming and require the special apparatuses, letting themselves performed only in limited numbers of clinics. Withdrawal of T4 in hypothyroid patients on T4 supplementation has sometimes been performed to determine whether they are truly hypothyroid [14, 17, 21]. There is a risk, however, for the development of significant hypothyroid symptoms. In contrast, measurement of RBC Zn concentration with a commercially available kit is highly reproducible [13] and easy to perform, and further omits the requirement of special apparatuses, exposure to radioactive substances and the risk for development of hypothyroidism.

Limitations include the small numbers of patients, especially in the TH group. We included only painless thyroiditis-associated hypothyroid patients, in whom preceding transient thyrotoxicosis had been confirmed, in the TH group. However, RBC Zn measurement may be useless if patients with painless thyroiditis develop PH following thyrotoxicosis despite of the fact that they rarely develop PH [12]. Further studies are accordingly required to evaluate RBC Zn levels in patients with various forms of hypothyroidism. In addition to thyroid dysfunction, several pathological conditions can affect the RBC Zn level; it is decreased in patients with severe anemia (Hb levels of less than 7.5 g/dL) but increased in pregnant women and patients with polycythemia or emphysema [22]. Our study did not include patients with such the conditions, however, attention should be paid to patient's complications to correctly evaluate their RBC Zn concentrations.

In summary, RBC Zn concentrations are significantly lower in painless thyroiditis-associated TH patients when compared with PH patients. Thus the measurement of RBC Zn is useful to distinguish TH from PH and to avoid unnecessary T4 administration to TH patients.

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