〈Original Article〉

Whole blood concentration of thiamine diphosphate requires correction for red blood cell count in the nutritional assessment of thiamine status

Hiroshi Ihara[1)], Toshikazu Suzuki[2)] and Naotaka Hashizume[2)]

Summary This study examined whether the whole blood (WB) concentration of thiamine diphosphate (TDP) needed to be corrected for hemoglobin concentration (Hb) or red blood cell (RBC) count. TDP levels in whole blood (WB TDP) and Hb concentrations and RBC counts were measured in 131 volunteers including subjects with anemia. WB TDP concentrations were more significantly correlated with the RBC count than the Hb concentration. Although the TDP/Hb and TDP/RBC values were significantly correlated with the WB TDP concentrations in the concordance studies, 14 subjects gave a discordant evaluation of thiamine status between assessment by WB TDP concentration and assessments by TDP/Hb value. Six of them revealed a discordant evaluation of the thiamine status between assessment by the WB TDP concentration and by the TDP/RBC value. All subjects with a lower WB TDP concentration presented with lower values of both TDP/Hb and TDP/RBC with or without the presence of anemia. While, the discordant cases were exclusively found in subjects having a normal WB TDP concentration (29-34 ng/mL: reference value, ≥ 29 ng/mL) but with lower values of TDP/Hb or TDP/RBC. Because these discordant cases were very often observed in correction by Hb concentration, the use of TDP/RBC values was recommended. We may conclude that the correction of the WB TDP concentration by the RBC count is necessary for the nutritional assessment of the thiamine status.

Key words: Vitamin B_1, Thiamine pyrophosphate, Anemia, RBC, Hemoglobin correction

1. Introduction

Vitamin B_1, an essential B vitamin, participates in the carbohydrate metabolism, in the tricarboxylic acid cycle and the pentose pathway. In cells, vitamin B_1 exists as free thiamine, thiamine monophosphate, thiamine diphosphate (TDP) and thiamine triphosphate. The principal biologically active form of thiamine is TDP. Measurement of the TDP concentration in red blood cell (RBC) provides a good indica-

[1)]Department of Laboratory Medicine, Toho University Ohashi Medical Center, 2-17-6 Ohashi, Meguro, Tokyo 153-8515, Japan
[2)]Department of Health and Nutrition, Wayo Women's University, 2-3-1, Konodai, Ichikawa, Chiba 272-8533, Japan
Correspondence to: Hiroshi Ihara,
Department of Laboratory Medicine, Toho University Ohashi Medical Center, 2-17-6 Ohashi, Meguro, Tokyo 153-8515, Japan
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tion of the adequacy of body stores, since the TDP level in RBC is depleted at a rate similar to that in other organs[1]. Recently, the TDP concentration in RBC has been reported in the whole blood (WB) corrected for the hemoglobin (Hb) concentration, because most of the total thiamine content of WB is in RBC as TDP[2, 3]. However, as there is no evidence that vitamin B_1 behaves as a cofactor in hemopoiesis, in the present study the TDP concentration corrected for the Hb concentration was compared with the TDP concentration corrected for the RBC count.

2. Subjects and methods

1. Subjects

Venous blood was collected from 131 self-reported healthy volunteers who were not taking a dietary supplement of vitamins. Study subjects were 24 men aged 37 to 41 y and 107 women aged 19 to 39 y. Written informed consent was obtained from all volunteers, and our study was in compliance with the rules for human experimentation at our institution.

2. Methods

TDP concentration in WB anticoagulated with Na_2EDTA was measured by a pre-column derivatization HPLC method using an NH_2-column as previously described[4]. We used anticoagulated WB (but not washed RBC) as specimens, since TDP in RBC was depleted during the washing process[4, 5, 6]. We used TDP-chloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as a standard, and the assay values were expressed as ng/mL in terms of TDP-chloride equivalent. The concentration of the prepared standard solution was checked by measuring absorbance at 248 nm[7]. We used 14.3×10^{-3} as the molar absorption coefficient of TDP-chloride in acidic solution (0.01 mol/L hydrogen chloride). In our previous report, reference value of whole blood TDP (WB TDP) concentration obtained from 509 healthy men and 460 healthy women was 70-229 nmol/L (32-106 ng/mL TDP-chloride equivalent) and 63-200 nmol/L (29-92 ng/mL TDP-chloride equivalent), respectively[8]. Hb levels and the RBC count were measured by an automated hematology analyzer (Cell-Dyn 3500R, Abbott Diagnostics Division, North Chicago, IL).

3. Statistics

Differences between men and women were analyzed by the Mann-Whitney U-test. Statistical significance was defined as $P < 0.05$.

3. Results

1. Relationship between WB TDP concentrations and Hb concentration, or RBC count

In this study, the WB TDP concentration for men ranged from 49 to 60 ng/mL (mean ± SD, 51 ± 5 ng/mL), and 20 to 61 ng/mL (mean ± SD, 43 ± 9 ng/mL) for women. Nine women were determined to have a vitamin B_1 deficiency (< 29 ng/mL), as estimated by the WB TDP concentration. WB TDP concentrations in men were all within the normal range (≥ 32 ng/mL). In this study, of the 107 women,

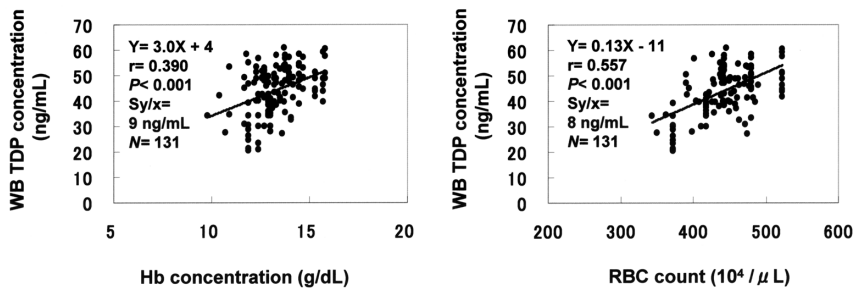

Fig. 1 Relation between WB TDP concentration and Hb concentration (left) and RBC count (right) in 131 volunteers.

23 were diagnosed with anemia (Hb, < 12 g/dL and/or RBC, < 380 × $10^4/\mu$L), while three women had a RBC count higher than the normal range (≥ 480 × $10^4/\mu$L). Both the Hb concentration and RBC count in men were within the normal range (Hb, ≥ 14 g/dL and RBC, ≥ 410-530 × $10^4/\mu$L).

When the WB TDP concentrations in all subjects were compared with their Hb concentrations or RBC counts, a positive relationship was observed between the WB TDP concentration and Hb concentration (r= 0.390, P< 0.001: Fig. 1, left). The RBC count correlated much more closely with the WB TDP concentration (r= 0.557, P< 0.001: Fig. 1, right). Naturally, the Hb concentration was significantly correlated with the RBC count (r= 0.798, P< 0.001, figure not shown).

2. Corrected WB TDP concentration

Because the WB TDP concentration was correlated with the Hb concentration and RBC count, the WB TDP concentration was corrected by dividing by the Hb concentration or the RBC count. In our subjects, the WB TDP concentration corrected for Hb (TDP/Hb value) ranged from 274 to 408 ng/g (mean±SD, 340±39 ng/g) for men, and 167 to 496 ng/g (mean±SD, 330±72 ng/g) for women. The WB TDP concentration corrected for RBC (TDP/RBC value) ranged from 83 to 122 × 10^{-10} ng (mean±SD, 102±12 × 10^{-10} ng) for men, and 55 to 141 × 10^{-10} ng (mean±SD, 98±19 × 10^{-10} ng) for women.

We selected from our 131 subjects 24 men and 58 women who were considered to have an adequate thiamine status with satisfying all the results of Hb concentration (≥ 14 g/dL for men and ≥ 12 g/dL for women), RBC count (≥ 410 × $10^4/\mu$L for men and ≥ 380 × $10^4/\mu$L for women) and WB TDP concentration (≥ 32 ng/mL for men and ≥ 29 ng/mL for women).

The 95% distribution range of TDP/Hb and TDP/RBC values was presumed from the above 24 men and 58 women. Because no significant gender differences were observed in the TDP/Hb value (mean±SD, 340±39 ng/g for men and 365±47 ng/g for women, P> 0.05) and TDP/RBC value (mean±SD, 102±12 × 10^{-10} ng for men and 109±14 × 10^{-10} ng for women, P> 0.05), the 95% distribution range was calculated without distinguishing men and women as the antilog of [(log mean)±2(log SD)], because the frequency distribution of TDP/Hb and TDP/RBC values were non-Gaussian, but log-normal. The 95% distribution range of TDP/Hb value was obtained as 272-462 ng/g, which was rounded to 270-460 ng/g, and the range of TDP/RBC value was 82-137 × 10^{-10} ng (rounded to 80-140 × 10^{-10} ng).

3. Concordance studies between WB TDP concentrations and TDP/Hb value, or TDP/RBC value

TDP/Hb and TDP/RBC values in 131 subjects were significantly correlated with the WB TDP

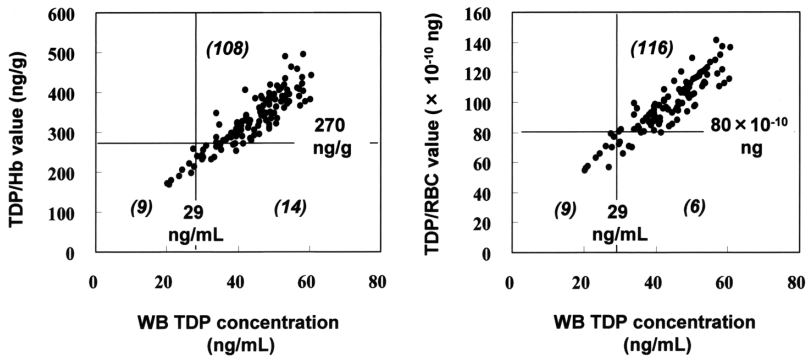

Fig. 2 Comparison between WB TDP concentration and TDP/Hb value (left) and TDP/RBC value (right) in 131 health volunteers. Vertical line indicates a lower cutoff value of WB TDP concentration, and horizontal line indicates the TDP/Hb value (left) and TDP/RBC value (right). Italic number in graph indicates the number of subjects (N).

concentrations. However, 14 female subjects gave a discordant evaluation of the thiamine status between assessment by WB TDP concentration and assessment by the above established 95% distribution range of TDP/Hb (Fig. 2, left). Six of them revealed a discordant evaluation of the thiamine status between assessment by the WB TDP concentration and by the TDP/RBC value (Fig. 2, right). Four were subjects with anemia and one showed an increased RBC count. In the remaining nine subjects, four had lower values of both TDP/Hb and TDP/RBC, and five had a lower TDP/Hb value and normal TDP/RBC value (Fig. 3). In the three women with an RBC count higher than 480 $\times$ $10^4/\mu$L, their WB DTP concentration and both TDP/Hb and TDP/RBC values were within the normal range, except one with a lower TDP/Hb value.

4. Discussion

Although there is no evidence that vitamin B_1 behaves as a cofactor in hemopoiesis except in thiamine-dependent megaloblastic anemia[9], we considered that the anemic subjects gave mistaken lower results of WB TDP concentration, because RBCs which contained TDP were often decreased in anemia. In this study, several subjects with anemia indeed showed a lower WB TDP concentration. Talwar[2] and Aasheim[3] corrected WB TDP concentration by Hb concentration, because the Hb concentration was significantly correlated with the RBC count. Talwar et al.[2] reported the TDP/Hb value to be 275-675 ng/g, which was similar to our cutoff concentration of 270 ng/g. Aasheim et al.[3] reported that men and women had different WB TDP levels but similar TDP/Hb values. In this study, we first found out that the RBC count correlated more significantly with the WB TDP concentration than the Hb concentration, and that no gender differences in TDP/RBC values.

Of the 131 subjects examined here including 23 anemic subjects, 14 subjects revealed a discordant evaluation of the thiamine status in the concordance studies between assessments by WB TDP concentration and its corrected value for Hb concentration. On the other hand, all subjects with lower WB TDP concentration represented lower values of both TDP/Hb and TDP/RBC with or without the presence of anemia. Discordant cases were exclusively found in subjects having normal WB TDP concentration but with lower values of TDP/Hb or TDP/RBC. The discordance was very often observed in correction by the Hb concentration than by the RBC count. Because TDP/RBC values are normal in some subjects, we thought that these discordances were artificially derived from Hb correction. Therefore,

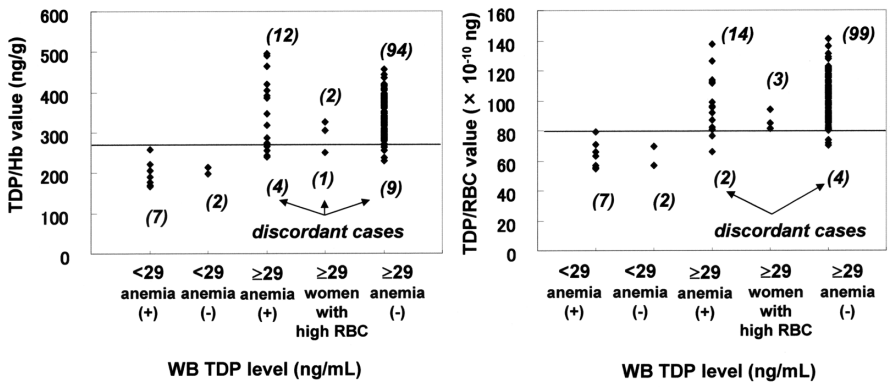

Fig. 3 Comparison between WB TDP concentrations and TDP/Hb value (left) and TDP/RBC value (right) in 131 volunteers with or without anemia. Row 4 composed of 3 dots indicates female subjects with high RBC. Horizontal line indicates a lower cutoff value of TDP/Hb value (270 ng/g: left) and TDP/RBC value (80 $\times$ 10^{-10} ng: right). Italic number in graph indicates the number of subjects (N).

TDP/RBC values were recommended rather than TDP/Hb values. The cutoff concentration of TDP/RBC value (i.e., 80×10^{-10} ng) would be the same between Japanese and other peoples of the world. We may conclude that correction of the WB TDP concentration by the RBC count is necessary for the nutritional assessment of the thiamine status.

References

1) Pearson WN: Blood and urinary vitamin levels as potential indices of body stores. Am J Clin Nutr, 20: 514-525, 1967.

2) Talwar D, Davidson H, Cooney J and St JO'Reilly D: Vitamin B_1 status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: comparison with erythrocyte transketolase activation assay. Clin Chem, 46: 704-710, 2000.

3) Aasheim ET, Hove AB and Bøhmer T: Thiamine assay values: influence of anticoagulants and haemoglobin. Scand J Clin Lab Invest, 69: 460-461, 2009.

4) Ihara H, Hashizume N, Hirano A and Okada M: A simplified HPLC method for thiamine and its phosphate esters in whole blood. J Anal Bio-Sc, 24: 235-238, 2001.

5) Lu J and Frank EL: Rapid HPLC measurement of thiamine and its phosphate esters in whole blood. Clin Chem, 54: 901-906, 2008.

6) Ihara H, Matsumoto T, Shino Y and Hashizume N: Assay values for thiamine or thiamine phosphate esters in whole blood do not depend on the anticoagulant used. J Clin Lab Anal, 19: 205-208, 2005.

7) Ihara H, Hashizume N, Hirano A, Wang L and Okada M: Purity of standard solutions of thiamine and its phosphate esters for HPLC analysis. J Anal Bio-Sci, 25: 272-274, 2002.

8) Ihara H, Hirano A, Wang L, Okada M and Hashizume N: Reference values for whole blood thiamine and thiamine phosphate esters in Japanese adults. J Anal Bio-Sci, 28: 241-246, 2005.

9) Alzahrani AS, Baitei E, Zou M and Shi Y: Thiamine transporter mutation: an example of monogenic diabetes mellitus. Eur J Endocrinol, 155: 787-792, 2005.