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Vitamin B_1 interpretation: Erroneous higher levels in non-anemic populations

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ABSTRACT

Objectives: The aims of this study were to underline the interpretation of vitamin B_1 and to evaluate whether differences in hemoglobin (Hb) levels and sex effect vitamin B_1 results.
Methods: Simultaneously, whole blood vitamin B_1 and complete blood count were determined in 2238 individuals. Groups were categorized on the basis of sex and Hb levels. Significance and correlation between groups and reference intervals of the study group were determined.
Results: There was an 8.4% ($P < 0.001$) difference between vitamin B_1 levels of men and women, whereas the ratio of vitamin B_1 to Hb showed a 0.12% ($P = 0.921$) difference. The reference interval for the ratio of vitamin B_1 to Hb was 268 to 675 ng/g Hb.
Conclusion: Vitamin B_1 concentrations >48 μg/L should be interpreted with Hb levels to avoid postanalytical errors that mask deficiency. Therefore, in comparative studies, researchers need to pay attention to eliminate the effect of Hb on whole blood vitamin B_1 levels.

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Introduction

Vitamin B_1 is an essential water-soluble vitamin that was first discovered in 1926 [1]. Together with its phosphorylated derivatives, vitamin B_1 plays a crucial role as a cofactor in enzymes and enzymatic complexes after esterification of the side-chain alcohol by a mono-, di-, or triphosphate [2]. Thiamine diphosphate is the most abundant biologically active form of vitamin B_1 in the human body, accounting for ~80% of the total vitamin B_1 content. Thiamine diphosphate is mainly found in leukocytes and erythrocytes, whereas plasma mainly contains free thiamine and its monophosphate ester [3]. Decreased levels of vitamin B_1 are found in many clinical manifestations, illustrating its importance. Some of these are related to various conditions and diseases such as traditional food-avoidance practices [4]; alcoholism [5]; drug interactions [6]; or several diseases such as metabolic syndrome [7], diabetes [8], obesity [9], critical illness [10], end-stage kidney disease [11], Alzheimer's disease [12], and postural tachycardia syndrome [13]. Because all forms of vitamin B_1 are important precursors that are involved in cellular energy metabolism and proper neuromuscular function [14], researchers began to investigate the connection between vitamin B_1 and the risk for mortality in patients in intensive care units [15] and in the diagnosis of Alzheimer's disease, using vitamin B_1 as a predictor of nutritional status [16].

Inadequacy of vitamin B1 in the diet, excessive intake of thiaminase-rich foods, or the occurrence of some pathologic processes can lead to reduced vitamin B_1 in the body [17]. Because of its very limited content in the human body and its high utilization rate, tissue thiamine stores may be depleted in up to 18 d in a healthy individual when subjected to a thiamine-deficient diet [18]. This refers especially to vitamin B_1 because the time to exhaustion of its stores is the shortest compared with all other nutrients [19]. It is obvious that the precise monitoring of vitamin B_1 deficiency and the diagnosis of subclinical deficiency have great importance.

In this context, the whole blood thiamine pyrophosphate (TPP) value is the most stable parameter in clinical chemistry and there is not much debate about its reference intervals. However, vitamin B_1 measurements have received little attention by the clinicians. Preanalytical and analytical variability of vitamin B_1 measurements are well established; however, interpretation of vitamin B_1 still contributes to postanalytical errors. Although interpretation of vitamin B_1 is well defined as ng/g hemoglobin (Hb) [20], most of the literature uses only vitamin B_1 levels without considering Hb values. Theoretically, using Hb levels in

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interpretation of vitamin B_1 may exclude the Hb effect because the majority of vitamin B_1 in the body is found in erythrocytes, accounting for ~80% of the total body store [21]. In clinical practice, Hb levels show higher values in men than in women. Therefore, this study aims to evaluate the effect of Hb levels and sex on B_1 vitamin interpretation.

Methods

Participants

This is a retrospective analysis of patients visited in 57 different clinics in the North Rhine-Westphalia state in Germany between January 1, 2016 and December 31, 2017. The study was carried out using data from Labor MVZ Dr. Stein + Kollegen (https://www.labor-stein.de/), one of the largest providers of laboratory testing in Germany. The patients were chosen from the outpatient part of the data set and test results stored in the laboratory information system were queried for the given time frame without any filtering. No results were excluded, and all of the results constituted the original database for this study.

The study was approved by the ethics commission located at the RWTH University Hospital Aachen. This authority provided a written statement that the study does not require any ethical vote. The respective authority argued as follows: The study as presented is covered by §6 of the law for the protection of personal data in the health care system (§ 6 GDSG NW), which allows data processing for scientific purposes. All data were analyzed anonymously preventing to draw conclusions on individual patients. In the federal state North Rhine-Westphalia this law is also valid after the introduction of the EU General Data Protection Regulation (EU-DSGVO) on May 25, 2018.

Analytical measurement

Parameters were determined in two laboratories: the Laboratory Diagnostic Center located at the RWTH University Hospital Aachen in Aachen and the motherhouse of the company which is the Medizinisches Versorgungszentrum (MVZ) Dr. Stein + Kollegen located in Mönchengladbach. Using the same methods by standardization in reagent lot numbers, calibrators, controls, and standard operating procedures significantly reduced the interlaboratory variability in the analyzed test results. Both laboratories participating in this study were standardized in this manner. Blood sampling was performed in a standardized manner by experienced health care professionals while patients were in a fasting state. Blood samples were collected in ethylenediaminetetraacetic acid tripotassium salt dihydrate (K_3-EDTA x $2H_2O$) evacuated plastic tubes. Hb levels were determined using the automated hematology system XN-9000 consisting of the analysis module XN 2000, the fully automated slide maker and stainer SP-10, and the high-end intelligent tube sorter for bar coded sample tubes TS 2000 (all from Sysmex Co., Kobe, Japan), which uses sodium lauryl sulphate in hemolysis procedure. The analytical performance of Hb had the following characteristics: analytical limits (0–26 g/dL), linearity (0–25 g/dL), coefficient of variation (CV; 3%), and accuracy (2%). Whole blood vitamin B_1 levels were measured as TPP. Analysis was performed by a high-performance liquid chromatography system consisting of a multisolvent pumping system (LC-20 AD-VP), equipped with a DGU-20 A5 degasser, an auto injector (SIL-20 A), and a CTO10 AS column oven (all from Shimadzu, Kyoto, Japan). The measurements were done with a RF-10 AXL fluorescence detector coupled to the chromatograph and an interphase module CBM-20 A system controller (all from Shimadzu). Analyses were carried out by commercial high-performance liquid chromatography reagent kit and column (Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany). The method was fully validated with performance characteristics of 92% recovery, 2 μg/L lower detection limit, and 720 μg/L linearity limit, respectively. Obtained precision expressed as the intra- and interassay coefficients of variations (CVs) were 1.3% and 2% for TPP-low concentration (24.2 μg/L), 2.1% and 1.7% for TPP-medium concentration (39.3 μg/L), and 0.9% and 2.3% for TPP-high concentration (93.6 μg/L), respectively.

Statistical analysis

Statistical analysis was conducted by using Microsoft Excel (Redmond, WA, USA) and SPSS version 18 (IBM, Ehningen, Germany). The different variables are reported as means ± standard deviation (SD), median, minimum, and maximum. Participants were classified by sex and Hb level. Continuous variables were expressed as means ± SD of the mean. A 95% confidence level was used and $P < 0.05$ was considered significant. Kolmogorov–Smirnov test was used to determine the normal distribution. Mann–Whitney U test was performed to determine whether different groups were statistically significant. Spearman correlation was used between Hb and vitamin B_1 levels. The Hoffmann method [22] was used to determine the reference intervals. The threshold for vitamin B_1 (T-TPP) requiring Hb correction was calculated with the following formula:

$$\text{T–TPP} = (\text{lower limit of the reference interval for vitamin } B_1/\text{Hb ratio}) \times (\text{upper limit of Hb reference interval})$$

Results

Descriptive properties of the study group and mean difference of parameters between sexes are presented in Table 1. The mean ages of the study groups were 60.9 ± 18.1 and 59.9 ± 19.6 y for men and women, respectively. In our cohort, 33% of women and 29% of men were anemic. No relationship was observed between age and vitamin B_1 levels ($r = 127$; $P > 0.05$). The correlation between vitamin B_1 with Hb and the ratio of vitamin B_1 to Hb were $r = 0.351$ ($P < 0.001$) and $r = 0.822$ ($P < 0.001$), respectively. There was an 8.4% ($P < 0.001$) difference between vitamin B_1 levels in the male and female groups, whereas the vitamin B_1-to-Hb ratio showed a 0.12% ($P = 0.921$) difference. The relationship of Hb with vitamin B_1 and the vitamin B_1-to-Hb ratio is presented in Figure 1A and B, respectively. The reference interval for the vitamin B_1-to-Hb ratio was 268 to 675 ng/g Hb with a mean of 472 ng/g Hb, whereas the threshold for vitamin B_1 (T-TPP) requiring Hb correction was determined as 48 μg/L. The quantile–quantile probability plot comparing the probability distributions of expected normal and observed values of TPP is depicted in Figure 2, and the diagnostic properties of tests with Hb levels are presented as Table 2. The difference between the corrected and not corrected vitamin B_1 values regarding different Hb levels are illustrated in Figure 3, and Hb values >14 g/dL were determined as a threshold to correct the vitamin B_1 levels.

Discussion

Many reviews clarified all aspects of vitamin B_1 about preanalytical, analytical, and postanalytic phases without emphasising the connection to Hb [23,24]. Even method harmonization studies do not take into account the importance of the vitamin B_1-to-Hb ratio in whole blood [25]. When we searched the literature from the past 34 y for the term *whole blood vitamin* B_1, we noticed only two reports that recalculated the levels of whole blood vitamin B_1 with the Hb concentrations [26,27]. In the present study, we found an 8.5% difference between men and women (Table 1) when not

Table 1
Description and comparison of participants grouped by sex

	Sex	n	Mean	SD	*P*-value*	Mean difference
Hb (g/dL)	Male	1072	14.1	2.1	<0.001	1.2
	Female	1166	12.9	1.6		(9.3%)
Vitamin B_1 (μg/L)	Male	1072	64.5	19.0	<0.001	5
	Female	1166	59.5	17.1		(8.4%)
Vitamin B_1-to-Hb ratio (g/μg)	Male	1072	464.6	158	0.921	0.006
	Female	1166	465.2	148		(0.12%)

Hb, hemoglobin; SD, standard deviation
*Difference between male and female.

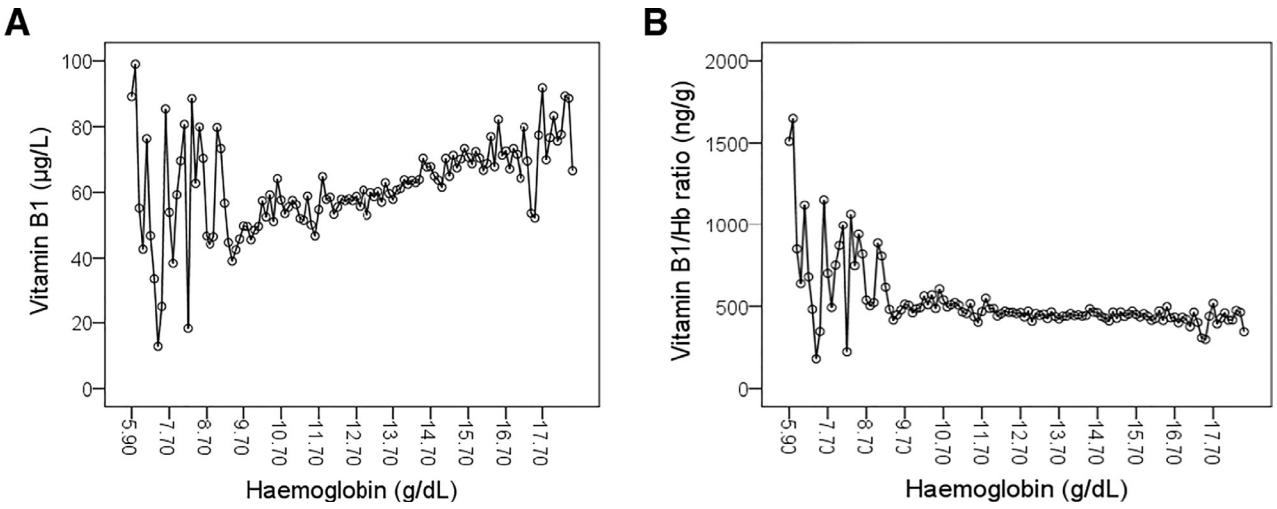

Fig. 1. Vitamin B and Hb. (**A**) Relationship between vitamin B_1 and Hb. (**B**) Relationship between vitamin B_1-to-Hb ratio and Hb. Hb, hemoglobin.

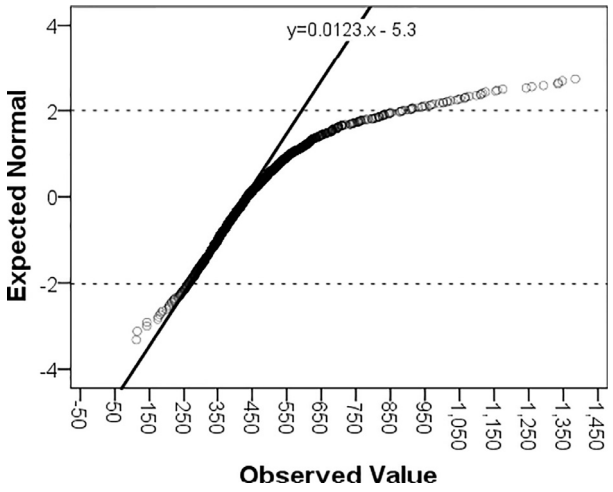

Fig. 2. Q-Q plot of expected normal and observed values of vitamin B_1-to-Hb ratios. Hb, hemoglobin; Q-Q, quantile–quantile.

Table 2
Diagnostic properties of tests*

		%	Mean Hb levels	SD
Low TPP values with normal TPP/Hb ratio, n	9	0.4	8.3	0.9
Low TPP values with low TPP/Hb ratio, n	27	1.2	11.3	1.8
Normal TPP values with low TPP/Hb ratio, n	29	1.3	14.1	1.3
Normal TPP values with normal TPP/Hb ratio, n	2173	97.1	13.5	1.9
Total, N	2238	100	–	–

Hb, hemoglobin; SD, standard deviation;TPP, thiamine pyrophosphate
*The conformity of the TPP with TPP/Hb ratio.

accounting for Hb concentrations. When considering Hb levels, minor differences were seen between the groups with a mean difference of only 0.12%. As a result, an 8.5% difference in the non-corrected group may give erroneous significance compared with the group corrected for Hb. This difference was presumed real (unaffected) by various authors even between the sexes [10,28].

Previously, it was suggested that there is a moderate positive relationship between vitamin B_1 and Hb [28]. A positive correlation between these two parameters is consistent with vitamin B_1 in the blood being mainly present in erythrocytes. In the present study, the same relation was observed (Fig. 1A). Based on this fact, it was stated that low whole blood TPP concentrations, which go along with anemia, should be interpreted with caution [29]. Some other studies pointed out that anemic patients with low TPP concentrations but normal vitamin B_1-to-Hb ratios can have adequate thiamine stores [30]. We observed the same situation in the present study group. Nine patients were falsely diagnosed with vitamin B_1 deficiency because of their anemic situation (Table 2). Therefore, interpretation of Hb levels or vitamin B_1-to-Hb ratio may prevent postanalytical errors in routine clinical laboratories.

We argue that TPP concentrations staying in the lower part of normal limits without presence of anemia should be interpreted in conjunction with Hb. Decreased whole blood vitamin B_1 values in patients with anemia showed erroneous lower levels that may result in the initiation of replacement therapy. But in the normal population, subclinical vitamin B_1 deficiency or insufficiency is masked by higher Hb levels if anemia is not present.

In this study, we determined that men who have higher Hb levels show erroneous higher vitamin B_1 levels than women. In contrast, the vitamin B_1-to-Hb ratio of both men and women was the same and consistent with the literature [30]. Moreover, excluding the effect of Hb makes the results more reliable. As shown in Figure 1, the vitamin B_1-to-Hb ratio was quite straight in the group without anemia. When considering that ~75% of the population consisted of non-anemic individuals, the statement becomes even more meaningful. Therefore, we concluded that vitamin B_1 concentrations <48 µg/L should be interpreted only in conjunction with Hb levels.

In the present study group, only 48% of patients (27 of 56) suffering from vitamin B_1 deficiency could be correctly diagnosed when we did not use the Hb correction (Table 2). Of the 56 patients, 29 (52%) of the vitamin B_1 deficiencies were masked by normal Hb levels. Figure 3 demonstrates that it is not possible to detect B_1 vitamin deficiency in individuals with Hb levels >14 g/dL. Therefore, vitamin B_1 levels in patients having higher Hb levels should be interpreted with caution.

In the present study, the determined reference intervals also confirmed previous reports. The lower limit of the vitamin B_1-to-Hb ratio (268 ng/g) was very close to those values reported by Talwar et al. (280 ng/g) and Ihara et al. (272 ng/g) [20,31]. All these studies suggest that comparing two or more groups, especially

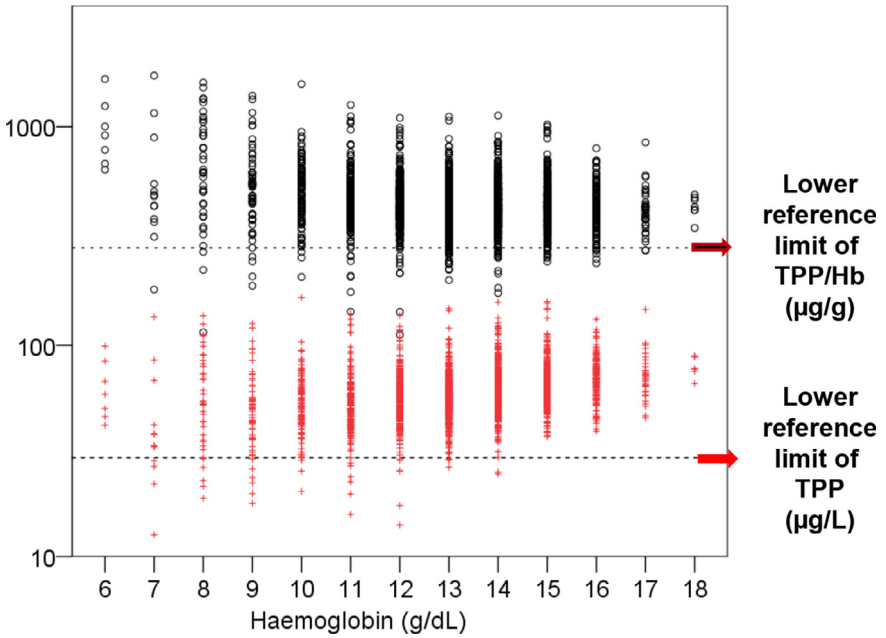

Fig. 3. Determination of lower reference limit of TPP and TPP/Hb in different Hb levels. Hb, hemoglobin; TPP, thiamine pyrophosphate.

when having different Hb levels, must consider the Hb ratios to exclude the anemia effect. One of these former studies further stated that correction of vitamin B_1 levels by the red blood cell is even better than correction for Hb and necessary for the nutritional assessment of the proper thiamine status [31]. However, in our view, the significance of this correlation is not high enough ($r^2 = 0.310$) to make clinical decisions. Because the preparation of washed red cells is time consuming, has safety implications, and increases the variability of results, we recommend the TPP in whole blood rather than in washed red cells be measured for the clinical laboratory assessment of the thiamine status [20].

Conclusion

We found that the interpretation of vitamin B_1 levels without Hb correction may lead to erroneously high results, thus masking the deficiency of the vitamin in non-anemic patients. Interpretation of vitamin B_1 status in patients with vitamin B_1 levels <48 µg/L or Hb levels >14 g/dL needs more caution. Clinicians and researchers should consider this fact to avoid misinterpretation of blood vitamin B_1 levels. However, one potential limitation of the present study was the lack of any clinical diagnosis of the patients with altered vitamin B_1 levels. In addition, we only applied one standardized method in the present study and possibly other methods might lead to slight variations.

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