



Technical Report

Effect of underfilling of tubes with EDTA on PTH assay measured by cobas analyzer

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Abstract

Objectives: The purpose of this study is to evaluate cobas parathyroid hormone (PTH) measurement effect of plasma samples obtained from underfilled tubes with ethylenediaminetetraacetic acid (EDTA).

Methods: Two blood collection tubes with K3-EDTA from 67 patients at the same time were taken. One of them was for routine PTH measurement, which was filled to its capacity, while another tube was underfilled. All EDTA tubes were immediately centrifuged at 4°C. Plasma PTH concentrations were measured by electrochemiluminescence immunoassay method on the cobas e 601 (Roche Diagnostics GmbH, Mannheim, Germany) analyzer.

Results: The underfilled sample tubes were grouped according to their being up to 25% (n=9), 25–50% (n=35), and 50–100% (n=23) of the appropriate amount. In all groups of underfilled tubes, the PTH values were found to decrease concerning those fulfilled ($p < 0.001$). The agreement among underfilled and fulfilled for measuring PTH demonstrated a bias of -3.2 pg/mL (-5.9%) for the underfilled tubes.

Conclusion: Insufficiently filled blood in tubes with EDTA has a significant effect on PTH levels. However, further studies are needed to show whether this effect is clinically significant.

Keywords: Parathyroid hormone, preanalytical phase, underfilled tubes

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Parathyroid hormone (PTH) is synthesized by the parathyroid glands, as an 84 amino acid full-length peptide being the biologically active form into circulation. There is not only the form of the full-length peptide in circulation but also multiple fragments. PTH results have several analytical and preanalytical variations due to being an unstable analyte and presence of fragments in the circulation [1]. Thus, many researchers have investigated the stability of PTH in different types of blood collection tubes. IFCC working group established for standardization of PTH assay recommended that blood samples for PTH measurement should be taken into tubes containing ethylenediaminetetraacetic acid (EDTA) [2]. The issue of inappropriate blood volume for anticoagulant tubes can be frequently seen in patients with poor venous access or small veins [3]. While the minimum sample volume is clearly defined for many tests, it is not available for PTH. Our aim for this study was to investi-

gate the effect of various proportions of underfilled EDTA-anti-coagulant blood tubes on PTH assay by cobas analyzer.

Materials and Methods

This study was performed in 1 week in the Clinical Biochemistry Laboratory of Zonguldak Bülent Ecevit University Hospital by approving of the Clinical Research Ethics Committee of Zonguldak Bülent Ecevit University Faculty of Medicine.

The research was carried out among the out-patients whose PTH levels were ordered to clinical chemistry laboratory. Sixty-seven participants were included in the study randomly, without applying any criteria. Venous blood samples were drawn according to the recommendations of the Clinical Laboratory Standards Institute, Document GP41 using the same personnel and materials in one blood collection center. Blood

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samples were collected in 2 mL K3-EDTA vacutainer tubes supplied by Becton-Dickinson (New Jersey, U. S. A.) with the same lot number (1069923). Two blood collection tubes with EDTA from each patient at the same time were taken, one of them was underfilled and another one was filled to capacity for routine PTH measurement. Therefore, each sample served as its own control in this experimental study.

The paired tubes were transported to laboratory at the same time and centrifuged at 3000 rpm for 5 min at +4°C. Plasma samples were analyzed for PTH levels immediately after centrifugation. Plasma PTH levels were measured by electrochemiluminescence (ECL) immunoassay method using cobas e 601 module (Roche Diagnostics GmbH, Mannheim, Germany). The assay range of the PTH is 1.2–5000 pg/mL and the within-run coefficient of variation of the PTH assay was <3%. The reference range for a PTH test is 15–65 pg/mL. Measurements were performed according to the manufacturer’s instructions using the same lot of PTH reagent, calibrator, and quality control sample. All internal quality control results were within acceptable ranges in the study period.

Statistical analysis

Statistical analyses were performed using SPSS® statistical software version 18 (SPSS Inc., Chicago, IL, USA). The normality of data distribution was accessed using the Shapiro–Wilk test. Data were analyzed by non-parametric method with Wilcoxon’s signed-rank test for paired samples. The biases of the results of PTH in underfilled tubes were assessed by calculating the percentage change from full as follows:

$$PTH_{\text{under}} - PTH_{\text{full}} / PTH_{\text{full}} \times 100$$

Bias analysis was performed using Microsoft Excel 4.0. The level of significance for all statistical comparisons was set as p<0.05.

Results

For 67 results obtained with underfilled tubes, mean values±SD of PTH level were 49.3±27.3 pg/ml (median 43.7 pg/ml) and for the corresponding fulfilled tubes, mean values±SD were found 52.5±29.4 pg/ml (median 44.3 pg/ml).

Underfilled samples were grouped according to filling the ratio of the sample as 0.5 ml and below (<25%), among 0.5 mL and 1.0 mL (25–50%), and among 1.0 mL and 2.0 mL (50–100%) (Fig. 1). The underfilled sample tubes comprised nine samples in below 25 %, 35 samples in 25–50%, and 23 samples in 50–100%. Comparisons of PTH levels between underfilled and fulfilled samples are presented in Table 1. In all groups of underfilled tubes, the PTH values were found to decrease concerning those fulfilled (p<0.001).

Furthermore, we have done bias analysis between paired tubes. The agreement among underfilled and fulfilled for measuring PTH demonstrated a bias of –3.2 pg/mL (–5.9%) for the underfilled tubes. The bias for PTH levels between the reference interval results (n=53) was –2.7 pg/mL, while the percent change in concentration was more pronounced (–6.2%).

Discussion

Our study showed significant differences between under and fulfilled samples for PTH. Similar results were reported in a study using the DPC Immulite [4]. The decrease in PTH levels in this study was attributed to the action in immunoassay



Figure 1. Grouping of underfilled samples.

PTH Levels	Paired blood tube volumes					
	<0.5 mL	2.0 mL	0.5–1.0 mL	2.0 mL	1.0-2.0 mL	2.0 mL
	n=9		n=35		n=23	
Mean concentration	50.2	53.6	49.0	52.8	49.3	51.6
Standard deviation	18.9	18.9	33.3	36.5	19.7	20.1
Median	46.6	50.6	37.5	41.3	44.5	49.0
Range	30.1–94.6	32.1–95.6	17.7–183	18.7–196	19.1–85	19.5–86
Concentration percentage change (%Bias)	–6.6		–6.5		–4.5	
p-value	<0.001		<0.001		<0.001	

PTH: Parathyroid hormone

methods that use alkaline phosphatase. Excess EDTA in underfilled tubes impairs activity of the alkaline phosphatase by chelation of metallic cations and can inhibit signal generation. Even if ECL immunoassays are not contained alkaline phosphatase enzyme, only a study on this subject in the literature showed that increasing EDTA concentrations resulted in the decline of hormone analyte levels in cobas analyzer [5]. It has been related that EDTA can change antigen conformation and the measurable analyte concentration [6, 7]. Although for under and fulfilled blood tubes with EDTA, the changes in PTH concentrations are statistically significant, the clinical relevance of the changes is questionable. Because, it is thought total error values of more than $\pm 30\%$ of the reference value will be clinically significant [8]. Negative bias values observed in our study did not exceed, 30% recommended by the CLIA or maximum error limit of ± 9.4 pg/mL for < 75.4 pg/mL reporting by Royal College of Pathologists of Australasia [9]. We also observed more bias% in normal values than in abnormal high values of PTH. However, the main limitation of our study, we could not compare between low levels of PTH in patients with hypoparathyroidism; thus, further studies are required. The second limitation is few and unequal numbers of available samples in all groups.

Conclusion

The present study suggests that insufficiently filled blood in tubes with EDTA have a significant effect on PTH levels. However, further studies are needed to show whether this effect is clinically significant.

Conflict of Interest: No conflict of interest was declared by the authors.

Ethics Committee Approval: The study was approved by the Zonguldak Bülent Ecevit University Faculty of Medicine Non-interventional Clinical Research Ethics Committee (No: 2022/04, Date: 23/02/2022).

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