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Stability of intact parathyroid hormone in serum and plasma samples stored at different conditions

Cigdem Yucel[1], Mujgan Ercan[2], Ahmet Rifat Balik[1], Murat Kizilgun[1]

[1]Gulhane Training and Research Hospital, Health Sciences University, Department of Medical Biochemistry,Istanbul, Turkey
[2]Afyon Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, Afyon, Turkey

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Abstract

Obtaining suitable results unaffected by preanalytical factors is crucial for laboratory test results. Measurement of parathyroid hormone (PTH) is essential in the evaluation and management of calcium metabolism disorders. The aim of the present study is to evaluate the effects of preanalytical factors such as sample type, storage time and temperature on PTH measurement in the same protocol. Blood samples were collected from 30 healthy volunteers into Serum Separator Tubes (SST), Barricor plasma tubes and EDTA aprotinine tubes. Serum and plasma were analysed immediately after collection simultaneously in the same batch and stored at room temperature RT (25ºC) and at (4ºC) for 24 and 48 hours until reanalysis for PTH on DXI800 autoanalyser (Beckman Coulter, USA). Changes in different tubes under different storage conditions were recorded and compared to each other. Clinical decision levels were estimated using analytical desirable bias and reference change value(RCV). No difference was found between tubes in terms of intact PTH levels. From a statistical standpoint, intact PTH level was no longer stable after storage for 24, and 48, hours at 4 C° and 25 C° in both serum tubes and barricor tubes. However, the variations especially in barricor tubes were under analytical desirable bias limits at 4 C^0 for 24 hours (-10.48%), and 48 hours (-15.42%) hours and the changes were also not clinically significant. Our results have shown that Barricor tubes are stable at 4 C° up to 48 hours.The stability of the measurements were both analytically and clinically acceptable.

Keywords: Barricor tube, preanalytical phase, parathyroid hormone, reference change value, storage conditon, serum separator tube

Introduction

Parathyroid hormone (PTH) is a 84 amino acid polypeptide hormone. PTH is stored in the parathyroid glands and released into circulation to regulate calcium and phosphate homeostasis through interacting with its receptors localised on the target cells in kidneys, bone and gastrointestinal system [1,2]. Measurement of PTH is critically important in diagnosis of serum calcium abnormalities and related metabolic disorders. Correct and accurate measurement of PTH has always been a challenge for laboratory specialists as there still is a lack of standardization and the hormone is prone to diurnal and seasonal variations and also staility issues [3].

Preanalytical phase is quiet important in clinical laboartories and almost 70% of total laboratory errors are known to occur during the preanalytical phase [4]. PTH is a peptide hormone and it's stability is low when compared to other biochemical parameters. Proteolytic cleavage through proteases affects peptide hormone levels, which is of particular significance when the time interval between sampling and analysis is prolonged. Other preanalytical factors affecting PTH can be listed as sample collection, type of the collection tube, sample type, transportation and storage conditions such as storage time and storage temperature [5,6]. The optimum procedure to measure intact PTH is rapid centrifugation after blood collection, followed by immediate measurement. This cannot always be guaranteed in many laboratories, due to factors such

*Corresponding Author: Ahmet Rifat Balik, Gulhane Training and Research Hospital, Health Sciences University, Department of Medical Biochemistry,Istanbul, Turkey E-mail: rifatbalik@hotmail.com

as test availability, equipment readiness, batching analysis, and distance from clinics to the laboratory, etc. Such delays could affect the stability of PTH, leading to measurement errors that may, in turn, affect the treatment of patients. Although the use of serum samples obtained with the use of gel separator tubes has almost become the industrial standard for spectrophotometric and immunochemistry analyses, the stability of peptide hormones in serum samples has been reviewed. In order to produce more accurate and clinically useful results, the stability of peptide hormones under the impact of handling procedures, storage conditions, and prolonged durations in different types of samples should also be investigated [7].

The aim of the present study is to evaluate the effects of preanalytical factors such as sample type, storage time and temperature on PTH measurement in the same protocol. Differences in PTH levels with different tubes and storage conditions will be evaluated according to total analytical errors (TEa) and reference change values (RCV), to emphasize if these changes are acceptable in terms of statistical significance, analytical performance specifications (APS) and clinical measures (RCV).

Materials and Methods

30 healthy adult volunteers were included in the study. Written consent was obtained from all participants and the study was approved by Harran University Clinical Research Ethics Committee (15.06.2020/11-HRU/20.11.03). In this research, the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals was complied with. Peripheral venous blood samples were collected from all participants in the same morning between 08.00-09.00 am in the morning after 8 hours of fasting, according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) document GP41. Three samples from each participant were colllected into the following tubes in a randomized order:

Tube 1: 6-mL BD vacutainer Serum Tube with clot activator (Ref number: 367819) (Becton Dickinson, New Jersey USA)

Tube 2: 5-mL BD vacutainer Barricor Tube- mechanical separation (Ref number: 365081) (Becton Dickinson, New Jersey USA)

Tube 3: 5-mL BD vacutainer EDTA/Aprotinine Tube glass tube with K3EDTA and protease inhibitör (Ref number: 361017) (Becton Dickinson, New Jersey USA)

All tubes were filled to capacity. Specimens were centrifuged at 3500 rpm for 10 minutes to obtain serum or plasma. The first measurement was carried out within 30 minutes from all specimens. The remaining samples were aliquoted into plastic tubes and stored at +4 C° and 25 C°for 24 and 48 hours.

All PTH measurements were done with DxI 800 immunoassay autoanalyzer (Beckman Coulter Inc).

Statistical Analysis

SPSS version 18 was used for all statistical analyses. Kolmogorov-Smirnov test was used for normality testing. Descriptive statistics were defined as mean ± SD or median (min-max). Statistical signifcance between groups were analyzed with ANOVA or Kruskal-Wallis test. PTH levels between study groups were compared with the analytical quality specifications for desirable bias, as obtained from the European Federation of Clinical Chemistry and Laboratory Medicine European Biological Variation Study. Additionally; for the clinically acceptable limit the reference change values (RCVs) were derived from within-subject biological variation and analytical variation. In the formula; RCV is calculated as $2^{1/2}xZx(CVA^2+CVW^2)^{½}$, where Z is the probability selected for statistical significance (Z=1.96 at 95% confidence interval, CI). CVA is the analytical imprecision. In the present study for CVA of the PTH test, analytical variation was estimated from analysis of internal quality control results. $p<0.05$ was accepted as statistically significant for all analyses.

Results

As the values were non-normally distributed, Kruskal-Wallis test was used to analyze difference between groups. There was no statistically significant difference in the initial measurements of PTH with 3 different tubes (Table 1).

Table 1. Comparison of Initial measurement values between three different tubes

Parameters	Tube 1 Median (min-max)	Tube 2 Median (min-max)	Tube 3 Median (min-max)	p values
PTH (pg/mL)	56.75 (16-289.5)	50.35 (15-288.2)	46.80 (11.8-245.5)	0.445

Tube 1: 6-mL BD vacutainer Serum Tube with clot activator; Tube 2:5-mL BD vacutainer Barricor Tube- mechanical separation; Tube 3:5-mL BD vacutainer EDTA/Aprotinine Tube glass tube with K_3EDTA and protease inhibitör, PTH: Parathyroid hormone

Changes in different tubes under different storage conditions were recorded and compared to each other. Values are expressed as median, 25-75 percentile and % change. The analytical and clinical acceptable changes were expressed as APS desirable bias and RCV. These values are also illustrated in Table 2. When we compared the values in Tube 1 stored in +4C° and 25C°, we found out that at both 24 and 48 hours; median PTH values tend to decrease also the changes were statisticallly significant ($p<0.001$ for both). The APS desirable bias value was 7.1% for PTH. At +4C°; the % change in 24 hours was -10.48 % while the %change in 48 hours was -15.42%. When Tube 1 was stored at RT (25C°); the % change values were -27.22% and -30.4% at 24 and 48 hours respectively. The calculated RCV for the present study is 46.6%. Our results have revealed that prolonged storage of Tube 1 at both RT and +4C° did not exert a % change over RCV value.

There was statistically significant differences after storage when compared to initial values with Tube 2. At +4C°; the % change results at 24 and 48 hours were -4.77%-6.06% respectively. But when tube 2 was stored at RT the % change was 12.91. Both storage at +4C° and 25C° were again within the RCV acceptable limits. The % change in Tube 2 in the first 24 hours at RT was -0.6.

Tube 3 was used as the reference tube and only stored at +4C°. The % change at 24 hour (h) was -5.56% and at 48 hwas -3,42% ($p<0.001$). The results are shown in Table 2.

Table 2. Comparison of changes in PTH measurement in different tubes and storage conditions

PTH (Serum tube)	Initial	24 hours	48 hours
4 ºC		50.8	48
Median	56.75	34.40-87.50	31.73-84.50
25.-75. Percentile	43.08-102.28	(-)10.48	(-)15.42
% Change		<0.001*	<0.001*
25 ºC			
Median	56.75	41.30	39.50
25.-75. Percentile	43.08-102.28	27.35-72.83	24.60-64.93
% Change		(-)27.22	(-)30.4
p value		<0.001*	<0.001*
PTH (Barricore)	Initial	24. hour	48. hour
4 ºC			
Median	50.35	47.95	47.30
25.-75. Percentile	36.85-89.45	33.33-86.55	34.50-77.13
% Change		(-) 4.77	(-)6.06
p Value		<0.001*	<0.001*
25 ºC			
Median	50.35	50.05	43.85
25.-75. Percentile	36.85-89.45	36.10-70.65	33.48-72.90
% Change		(-) 0.6	(-) 12.91
p Value		<0.001*	<0.001*
PTH (Aprotinin)	Initial	24. hour	48. hour
4 ºC			
Median	46.80	44.20	45.20
25.-75. Percentile	34.65-86.90	32.63-77.03	29.98-74.18
% Change		(-) 5.56	(-)3.42
p value		<0.001*	<0.001*
APC (Desirable Bias)	7.1	7.1	7.1
RCV	46.6	46.6	46.6

PTH: Parathyroid hormone, RCV: Reference change value

Discussion

When the initial measurements of PTH were compared between three tubes; no statistically significant difference was detected. This may be accepted as a confirmation for the serum tubes (Tube 1) we use in our routine analysis procedure when compared to the reference tubes (Tube 3) with protease inhibitors. Similar to our study, Khalil et al. compared the measurement of intact PTH in Abbott Architect analyzer at different tubes and storage times. They have used serum tubes, SST tubes and EDTA tubes. No statistically significant difference was found between PTH measurements [8]. In another study; Immulite 2000 analyzer was used to compare measured PTH levels between serum and EDTA plasma samples [9]. This study showed that there was a significant difference in PTH mesaurements between these tubes. These differences may show us that PTH results can be affected by analysis method and preanalytical factors like tube types.

Baykan et al carried out a study with 10 healthy volunteers in serum tubes with 2 sets of tubes under different storage conditions (RT and 4°C) and up to 6, 24, 48, and 72 hours. In that study; PTH levels were stable at RT statistically but at 48. hours, analytical

bias limit was exceeded. However at 4°C, the results were still under the analytical bias limit [10]. On the contrary; Khalil et al have demonstrated that PTH results were different when compared to basal measurements in both serum and plasma samples when stored at both 25°C and 4°C. Although the differences were statistically significant, when evaluated in terms of total allowable errors , the serum samples stored at RT had exceeded the 30% TEa at all storage times but plasma samples were stable in terms of TEa. This marks that the plasma samples were more stable than serum samples. When stored at 4°C , both changes in serum and plasma samples were under 30% TEa level [8]. In our study, we found out that plasma samples obtained by Barricor tubes are more stable than serum samples under prolonged storage conditions both analytically and clinically as we evaluated RCV.

Tube 2 (Barricor tubes) provide mechanical separation. There was statistically significant differences after storage when compared to initial values. But especially at +4C°; the % change results at 24 and 48 hours were both under the APS acceptable bias limit. This makes these storage conditions also analytically acceptabele. But when tube 2 was stored at RT; it exceeded the APS limit. Both storage at +4C° and 25C° were again within the RCV acceptable limits, which makes this tube clinically acceptable. The % change in Tube 2 in the first 24 hours at room temperature was as low as -0.6%. These data may suggest that Barricor tubes can be stored at either RT or refrigerated for 24 hours and it meets both analytical and clinical quality criteria.

Tube 3 was used as the reference tube in this study as it contained protease inhibitör which is quite important in the prolonged stability of PTH. We stored these tubes only at +4C° because storage at RT is not recommended and we stored samples regrigerated to provide the ideal environment for storage. The prolonged storage in this tube showed stable results in terms of statistical significance, analytical quality measures (APS) and clinical measures (RCV).

The upfront feature of our study is the inclusion of RCV in the stability criteria. Most of the previous studies have evaluated stability according to analytical bias or TEa while we also evaluated clinical significance by evaluation of RCV. This is the first study in the literature that includes RCV in the stability of PTH in different tubes under prolonged storage conditions. The major limitation of our study is that we did not stored the reference tube (Aprotinin tube) at room temperature, only storage up to 48 hours at +4C° was done for the reference tube so we did not have the chance to evlaute the PTH level changes in the reference tube at RT.

Conclusion

Our results have shown that Barricor tubes are stable at 4C° up to 48 hours. When compared to the routine serum tubes we use (Tube 1), Barricor tubes can be a good alternative as they provide a superiority when the samples are stored at 4C° up to 48 hours and they can be used as an alternative to red-capped serum tubes.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Written consent was obtained from all participants and the study was approved by Harran University Clinical Research Ethics Committee (15.06.2020/11-HRU/20.11.03).

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