

# Comparison of Stability of PTH in Serum and in EDTA Plasma

IMRAN ALI, ASMA SHAUKAT, LUBNA SARFRAZ, MUHAMMAD WASEEM

## ABSTRACT

**Aim:** To determine the mean difference in concentration of PTH in EDTA plasma and serum at room temperature (22°C).

**Design:** Randomized control trial.

**Setting:** Department of Chemical Pathology, Quaid-e-Azam Medical College Bahawalpur.

**Methods:** 560 volunteers were randomly divided into two groups A and B. To group A blood sample EDTA was added, whereas, blood samples from group B were analyzed without adding EDTA. About 10 ml of blood was drawn from each patient and serum was separated within 30 min by centrifuge machine. The serum was then divided into three aliquots, to be analyzed as base line reading and at 24 and 72hrs.

**Results:** Samples without preservative showed statistically significant decrease in PTH concentration from baseline after 24hrs ( $p < 0.0001$ ) and at 72hrs ( $p = 0.0001$ ). Whereas the EDTA samples were stable at 24 hrs ( $p = 0.32$ ) and 72 hrs ( $p = 0.64$ ).

**Conclusion:** Use of EDTA plasma for PTH measurement greatly increases PTH stability even at room temperature.

**Keywords:** Parathyroid hormone, stability, EDTA, preanalytical error.

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## INTRODUCTION

Measurement of intact parathyroid hormone (PTH) is essential to the diagnosis and management of metabolic bone disease, hypercalcaemia, hypocalcaemia and renal dystrophy.<sup>1</sup> The effect of specimen type, collection temperature, and storage temperature on the in vitro stability of PTH differ by method and platform<sup>1</sup>. Characterization of preanalytical effects unique to each method, platform, and patient population is important to prevent potential clinical misdiagnosis<sup>1</sup>.

PTH is a peptide hormone hence it is susceptible to proteolytic degradation by enzymes which exist in tissues and extracellular fluid so if there is considerable time lapse between sample withdrawal and analysis, when no preservative is used to halt such proteolytic changes, then the concentration of such hormones can decrease significantly with time.

Quality of a test performed in clinical laboratory does not merely depend on analytical aspects<sup>1</sup> but it also needs much care regarding sample handling, time lapse between drawing a sample and performance of required test, storage temperature and added preservatives<sup>3</sup>.

Recent research studies show that most of the laboratory errors (32% to 75%),<sup>4</sup> are contributed by preanalytical phase<sup>5</sup>, so it is the need of time to

improve this sector of laboratory procedures to yield better results<sup>6</sup>.

In a study done at Armed Forces Institute of Pathology (AFIP) Rawalpindi the values of thyroid stimulating hormone (TSH)<sup>7</sup> and PTH<sup>8</sup> were found statistically different ( $p < 0.05$ ) after 24hrs (value PTH=11.3, TSH=0.99) and 72hrs (value PTH=7.3, TSH=0.33) respectively from 0hr (value PTH=12.7, TSH =1.3) with regard to duration and added preservatives.

In a study conducted on PTH at Royal Perth Hospital, Western Australia, statistical analysis of all results indicated that serum values both at baseline and after three days were statistically lower than the target value ( $P < 0.0001$ ). Serum PTH value after three days of storage at room temperature was >60% lower than the baseline sample whereas EDTA sample PTH value was marginally higher (8%)<sup>9</sup>.

Quaid-e-Azam Medical College Pathology laboratory, being a tertiary care laboratory, provides diagnostic facilities to the entire district and specimens from peripheral laboratories take 1-2 days to reach the QAMC laboratory. This time lapse shows a need to increase the stability of specimens, thus allowing for transportation of samples from remote areas to central laboratory.

Stability of samples is a big issue in case of PTH especially if it reaches the laboratory after a significant time lapse. Outcome of this study helped in estimating the mean difference in concentration of PTH in samples with and without preservative and

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*Pathology Department, Quaid-i-Azam Medical College, Bahawalpur, Pakistan*

*Correspondence to Dr. Imran Ali, Demonstrator Email: doctorimranali@yahoo.com*

also in creating awareness that the use of EDTA can significantly increase the stability of PTH.

## MATERIAL AND METHODS

This randomized control trial was conducted in the Department of Chemical Pathology, Quaid-e-Azam Medical College Bahawalpur from October 1, 2012 to May 30, 2013. Taking the mean  $\pm$ SD for the two groups  $12\pm 3.75$  and  $11.3\pm 3.68$  with 80% power of the test and 95% confidence interval, 280 subjects were included in each group, making a total of 560 patients. Sampling technique was non-probability consecutive sampling. All patients coming for thyroid hormone analysis between age of 20-60 years of either sex (as study was designed to determine the mean difference in concentration of PTH from first reading, so age, sex, diseased or non diseased condition were not to effect the stability of PTH) were included in the study. Patients who were not willing to give sample of 10ml of blood and Haemolysed or lipaemic samples were not included in the study.

Data collection was started after approval of the study by the local ethics committee and permission from the Head of Pathology Department. Purpose of the study was explained to the patients visiting pathology laboratory for thyroid hormone analysis. After taking informed consent from patients meeting inclusion criteria, they were randomly divided into two groups A and B, by lottery method. To group A blood sample EDTA was added, whereas, blood samples from group B were analyzed without adding EDTA. About 10ml of blood was drawn from each patient and serum was separated with in 30 min by centrifuge machine. The serum was then divided into three aliquots. One of the aliquots was analyzed within one hour after collection and the value obtained was considered as zero reading or base line reading. The second and third aliquots were analyzed after 24hrs and 72 hrs respectively. All the samples were analyzed at room temperature on VITROS ECi/ECi Q System using Chemiluminiscence

technique by a medical technologist with 10yrs of experience. The researcher had borne all the expenses. Measurement bias was controlled by frequent calibration of machine and repeating each test twice and taking the mean of the readings.

Data was entered and analyzed through SPSS version 15. Frequency and percentage was computed for categorical observation like gender. Mean and SD was calculated for PTH values at base line, 24hrs and 72hrs, with and without EDTA. Concentration of PTH with and without EDTA was compared between groups using student t-test. P-value  $\leq 0.05$  was taken as significant. As this study was to determine the mean difference in concentration of PTH from the first reading, so age, gender and PTH levels were not confounding variables and stratification was not done on their basis.

## RESULTS

In this study samples from 560 subjects were obtained. 50% of the study participants were males. The mean PTH concentrations of each sample type are shown in Table 1 and figure3. The results of the study showed that the serum samples analyzed after 24hrs from the collection time showed statistically significant difference in their mean concentration value ( $p < 0.0001$ ) from the baseline value. The difference is about 14% decrease in mean PTH concentration. When the samples were analyzed at 72 hrs after collection, they showed decrease of about 45.6% in mean concentration ( $p = 0.0001$ ). This is an extremely significant decrease. To the samples from group B, EDTA was added. Their analysis at 24hrs showed an increase in PTH concentration which was not statistically significant. The 72hrs samples showed a little decrease in PTH concentration which was again higher than the baseline. These differences again were not statistically significant.

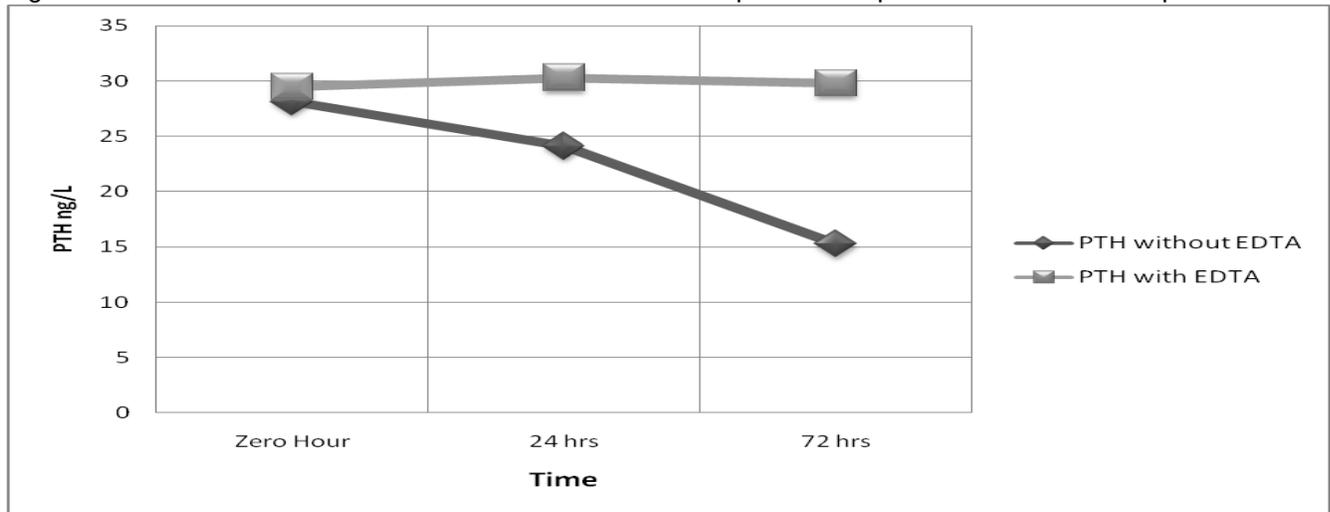
Table 1: Mean and SD (standard deviation) Of PTH results in ng/L

Sample type	Baseline (zero hour)		24 hours		72 hours	
	Mean	SD	Mean	SD	Mean	SD
Serum	28.1	9.2	24.1	8.3	15.25	5.6
EDTA Plasma	29.5	9.4	30.3	9.5	29.8	9.4

Table 2: Mean and standard deviation of difference in PTH concentration in ng/L with regard to time lapse.

Sample type	Baseline (zero hour)		24 hours	
	Mean	SD	Mean	SD
Serum	3.95	0.8	12.80	4.4
EDTA Plasma	0.8	0.42	0.37	0.36

Fig. 3: Fall in mean PTH concentration with time in serum samples as compared to those of EDTA plasma.



## DISCUSSION

Stability of PTH hormone has always been an issue and various studies have been conducted to find out ways to improve its stability and to find out how long the hormone stays stable with a certain preservative. The results of this study are broadly in keeping with those of other studies and the slight differences may be due to the fact that we used non uraemic subjects with normal PTH values.

Newman et al<sup>10</sup>, showed that PTH concentration decreases by 14% over 18hrs in serum and a study by Levin et al showed that the concentration of PTH decreased by 47% over 72hrs but did not decrease in EDTA plasma<sup>11</sup>. These results are very much similar to our study results. Ratcliffe et al<sup>12</sup> observed decrease of 17% in serum PTH over 24hrs. Russel and Henley showed a 30% decrease in measured PTH in serum at room temperature after 24 hrs<sup>13</sup>. Teal et al<sup>14</sup>. showed significant decrease in serum samples after 24 and 48 hours, however their EDTA plasma samples showed a rise in PTH sample by 24hrs and a slight decrease by 48 hrs which is still a little higher than base line value. These differences were statistically insignificant. Same trend was observed in our study. Walker and Seth also found that Concentrations remained stable in EDTA plasma<sup>15</sup>. Gladening et al.<sup>16</sup> found that after three days of storage, measured PTH concentration in EDTA had not decreased, but had declined by more than 40% in serum. Omar et al<sup>17</sup> also observed improved stability in EDTA plasma.

Our study also found statistically significant decrease in PTH concentration over 48 and 72 hrs and a slight statistically insignificant increase in PTH concentration in EDTA plasma from base line value

which is similar to the findings of studies by Teal et al. and Gladening et al.

Differences between the results of various studies may be due to different assay principles and study populations. There may also have been subtle differences in sample handling and preparation.

## CONCLUSION

This study shows that PTH concentration stays stable in EDTA plasma for about 72 hrs at room temperature where as it falls in serum without preservative, giving false low results. Thus use of EDTA plasma for PTH measurement greatly increases PTH stability even at room temperature. This greater stability allows for transporting samples from remote areas to central laboratory.

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