Stability of Parathyroid Hormone in Serum versus Plasma in Patients with Chronic Kidney Disease

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ABSTRACT

Objectives: To determine the stability of parathyroid hormone in patients with chronic kidney disease both in serum and ethylenediaminetetraacetic acid (EDTA) plasma tested at room temperature.

Methods: This is a prospective study conducted at Princess Iman Centre for Research and Laboratory Sciences, King Hussein Medical Center Jordan. A total of 94 patients were included with age range between 10 years and 65 years, of which 42.5% male and 57.5% were female.

From each patient 5 ml EDTA acid and 10 ml gel separator (with clot activator) tubes were collected and analyzed in a time period between 1/7/2015 and 1/11/2015. The plasma and sera were analyzed for intact parathyroid hormone within 6 hours of collection (baseline) and after 48 hours stored at room temperature, using immulite 2000 intact parathyroid hormone assay. Mean parathyroid hormone value, median, and differences for sera and EDTA samples at baseline and after 48 hours of collection were calculated and evaluated using Microsoft excel sheet.

Results: Serum samples analysed after 48 hours from the collection time showed significant difference in their mean concentration value from the baseline mean value. Regarding EDTA acid plasma samples, their analysis after 48 hours showed a little decrease in parathyroid hormone concentration from the baseline value, which was not significantly different. The percentage of difference between baseline mean value and 48 hours mean value was 7.91 % for EDTA acid plasma samples and 11.1% for serum samples.

Conclusion: The study showed that the parathyroid hormone was more stable in EDTA acid plasma sample than serum at baseline and after 48 hours of collection at room temperature. Serum samples gave inaccurate and false low results. Therefore, plasma measurement for parathyroid hormone analysis is preferred and advised. However, reference range for PTH concentration in plasma should be established if it is needed to be adopted for routine clinical use.

Key words: parathyroid hormone stability, processing temperature, chronic kidney disease

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Introduction
Parathyroid hormone (PTH) is a single-chain polypeptide, comprising 84 amino acids. It is synthesized as a larger precursor (pre- and pro-PTH) in the parathyroid glands in response to reduction in plasma calcium concentration, and secretion is inhibited by hypercalcemia. PTH has calcemic and phosphaturic biological responses in kidney and bone. (1) In the kidney, PTH increases calcium reabsorption, decreases reabsorption of phosphate, and increases production of 1, 25 (OH) vitamin D, which stimulates intestinal absorption of both calcium and phosphate. In the bone, the PTH stimulates bone, resorption. Also it increases total and

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free calcium, decreases plasma phosphate.\(^{(2)}\)

In chronic kidney disease, the defect in calcium and phosphorus homeostasis is an important consequence that might lead to increase in serum PTH and abnormal calcium and phosphorus levels. These abnormalities are considered part of bone and mineral disorder related to chronic kidney disease. The National Kidney Foundation Kidney Disease Outcome Quality Initiative (K-DOQI) guidelines recommend that PTH, calcium, and phosphorus should be measured in patients with bone and mineral disorder related to chronic kidney disease.\(^{(3)}\) Determination of PTH is helpful in the differential diagnosis of hypocalcaemia and hypercalcaemia, assessment of parathyroid glands function in kidney failure and evaluation of parathyroid function in bone and minerals disorders.\(^{(4)}\)

The majority of samples received for PTH testing come from patients with chronic kidney failure, in whom PTH was used as a marker of bone turnover.\(^{(5)}\) Several analytical and pre-analytical conditions are important for good evaluation of PTH results. Routine testing of PTH in our lab is performed using serum samples, which might be an alternative to EDTA plasma. However, the stability of PTH in EDTA plasma and serum are different and represents one of the important pre-analytical factors.\(^{(7)}\)

In the present study the stability of PTH in plasma and serum samples will be evaluated.

**Method**

The study was approved by ethics committee of the Royal Medical Services, Amman-Jordan. Study was conducted at Princess Iman Centre for research and laboratory sciences in a time period between 1/7/2015 and 1/11/2015 in a prospective manner. A total of 94 patients were included with age range between 10 years and 65 years, 42.5% of which male and 57.5% female.

Blood samples from all patients tested were collected in parallel into 5 ml EDTA and 10 ml gel separator (with clot activator) tubes. All tubes were filled as indicated by the label on the tube and referred to the laboratory within 30 minutes. They were centrifuged at room temperature, and the plasma and sera were collected in cap-closed tubes. Lipemic, haemolysed and icteric samples that may give erroneous results were excluded from the study. The plasma and sera were analysed for intact PTH within 6 hours of collection and after 48 hours storage at room temperature.

PTH was assayed using immulite 2000 intact PTH assay with lot PIL2KPP-19, which is a solid phase, two site chemiluminescent enzyme labeledimmunometric assays. Data including patients name, sex, national number, parathyroid hormone concentration were entered and analysed using Microsoft excel sheet, windows 7 home premium. Mean PTH value, median, and differences for sera and EDTA plasma samples at baseline and after 48 hours of collection were calculated.

**Results**

In our study we compared the PTH concentration in EDTA plasma and serum from 94 patients with chronic kidney disease at baseline and after 48 hours at room temperature. According PTH levels patients were divided into 3 groups: Group A (8 patients) with normal PTH level (15-65 pg/ml), Group B (86 patients) with high PTH level (> 65 pg/ml), Group C (no patients) with low PTH level (< 15 pg/ml).

Plasma and serum PTH concentration ranged from (14.7-1908 pg/ml) and (8.14-1490 pg/ml) respectively, the PTH concentration were higher in EDTA plasma samples than in serum (Table I). Serum samples analysed after 48 hours from the collection time showed significant difference in their mean concentration value from the baseline mean value. However, EDTA plasma samples results showed a little decrease in PTH concentration after 48 hours from the baseline value, which was not significantly different (Table II and figure 1).

The percentage of difference between baseline mean value and 48 hours mean value was 7.91% for EDTA plasma samples and 11.1% for serum samples (Table III).

**Table I:** Plasma and serum PTH concentration range at baseline and after 48 hours of collection.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>PTH CONCENTRATION RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASELINE</td>
<td>15.1-1908 pg/ml</td>
</tr>
<tr>
<td>48 HOURS</td>
<td>14.7-1805 pg/ml</td>
</tr>
</tbody>
</table>
Table II: the mean value of plasma and serum PTH concentration, baseline and after 48 hours of collection in pg/ml

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Baseline (mean)</th>
<th>After 48 hours (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLASMA</td>
<td>287.46</td>
<td>264.75</td>
</tr>
<tr>
<td>SERUM</td>
<td>243.5</td>
<td>197.05</td>
</tr>
</tbody>
</table>

Figure 1: comparison of decrease of PTH concentration in plasma and serum.

Table III: difference in mean value for EDTA plasma samples and serum

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Difference in PTH concentration in difference(pg/ml)</th>
<th>Percentage of difference(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLASMA</td>
<td>22.71</td>
<td>7.91 %</td>
</tr>
<tr>
<td>SERUM</td>
<td>46.45</td>
<td>11.1 %</td>
</tr>
</tbody>
</table>

Discussion

Stability of PTH is an important factor for precise and accurate results. Routinely, Samples requested for PTH testing are collected at in campus clinics and off campus peripheral hospitals and then sent to our centre (Princess Iman Centre for Research and laboratory sciences) for analysis. These clinics and hospitals are remote from the centre where samples are spun at room temperature. Therefore, it is important to know and reduce the variability to a maximum in the handling, processing, and storage of samples in order to keep the pre-analytical errors to the minimum. Our results showed that the concentration of PTH was lower in serum when compared with EDTA plasma either at baseline or after 48 hours of collection. There are many studies compared the stability of PTH in EDTA and serum tubes. Several studies showed that the stability of PTH in EDTA plasma was higher than serum. Imran Ali et al., (8) showed that the serum samples analyzed after 24 hours and 72 hours from the collection time showed statistically significant difference in their mean concentration value. The differences about 14 % (after 24 hours.) and 45.6% (after 72 hours.) decreased in serum mean PTH. Levin et al,(9), showed that the PTH concentrations was stable when blood was collected in heparin, EDTA and protease inhibitor tubes during the 48 hours period at room temperature, except in one occasion with markedly elevated plasma amylase activity. Teal et al,(10), showed the PTH was stable in EDTA plasma after 48 hours at room temperature, whereas the serum PTH concentration decreased after 2 hours and 4 hours at room temperature. Moreover, Morales Garcia and his colleagues showed the stability of PTH in EDTA plasma samples at room temperature as well as if samples froze immediately after centrifuging. However, a study conducted at Southampton General Hospital, UK found EDTA plasma results to be significantly higher than those in serum with an average increase of 19.5% over the serum result, nevertheless, results from EDTA plasma samples left to stand at room temperature for 48 hours were on average 14.8% lower than results from the corresponding EDTA plasma samples frozen within 30 minutes. The results of our study showed that the difference between PTH mean values was higher in serum than EDTA plasma samples analyzed at baseline and after 48 hours of collection. Finally the differences among various studies may be due to assay procedure, sample processing, and study population.

Conclusion

The study showed that the PTH was more stable in EDTA acid plasma sample than serum at baseline and after 48 hours of collection at room temperature. Serum samples gave an inaccurate and false low result. Therefore, plasma measurement for PTH analysis is the preferred method. However, reference range for PTH concentration in plasma should be established if it is needed to be adopted for routine clinical use.
References


