Comparison of intact parathyroid hormone (iPTH) assays in the diagnostic laboratory: Roche Modular E170 and Advia Centaur

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Abstract

Background: Parathyroid hormone (PTH) is synthesized in parathyroid glands and regulates plasma ionized or free calcium (Ca\textsubscript{i}). It also has an effect on the regulation of free phosphate (Pi). The biologically active site of intact PTH (iPTH) is found in the N-terminal region 1-34.

Objective: The level of iPTH can be measured using non-competitive immunoassay or on both Roche Modular E170 and Advia Centaur analyzers. The objective of this study was to compare the iPTH results from both analyzers to identify whether they provided interchangeable results.

Methods: K3-EDTA plasma samples from 24 patients were collected and analyzed in duplicate on both Roche Modular E170 and Advia Centaur analyzers.

Results: The paired duplicate data from the Roche Modular E170 and the Advia Centaur gave the internal reliability (CV) of 7% and 3%, respectively. The correlation coefficient (r^2=0.995) showed good association between the two analyzers, however, the Bland and Altman difference graph demonstrated a significant increase in mean difference in higher iPTH concentrations with the Advia Centaur compared to the Roche Modular E170 (t-test: 3.77; p<0.05).

Conclusions: The iPTH assay on the Advia Centaur showed better internal reliability compared to the Roche Modular E170. Despite the requirement of high plasma volume, the Advia Centaur can be the analyser of choice to perform iPTH assay.

Key words: intact parathyroid hormone, non-competitive immunoassay, electrochemiluminescence, chemiluminometric, Advia Centaur, Roche Modular E170


Introduction

Parathyroid hormone (PTH) is synthesized by four parathyroid glands which are located close to or on the posterior surface of the thyroid gland. However, additional parathyroid glands may be located elsewhere such as in the neck or within the thymus in the superior mediastinum (1). PTH is a single-chain polypeptide of 84 amino acids with molecular weight of 9500 Daltons. Intact PTH, iPTH, which is the form that is secreted into the blood stream, is less than 10 minutes on which iPTH is cleaved in the region of the liver and kidney, to an amino acid (N-terminal) fragment of at least 34 amino acids, carboxy-terminal (C-terminal), and mid-peptide fragments (1, 2). The biological activity of iPTH resides in the first 34 amino acids of the N-terminal, therefore, both iPTH and C-terminal fragments possess full biological activity.

Together with vitamin D and calcitonin, iPTH binds to type 1 PTH receptors on target tissues and regulates the ionized or free calcium (Ca\textsubscript{i}) (1). The level of iPTH also affects the concentration of plasma phosphatase (Pi), even though the plasma Pi concentration does not control iPTH secretion directly. Elevated plasma iPTH which causes hypercalcaemia is found in primary hyperparathyroidism and secondary hyperparathyroidism. On the other hand, low plasma iPTH, which causes hypocalcaemia, is found in primary hypoparathyroidism and secondary hypoparathyroidism.

The plasma concentration of iPTH can be measured by non-competitive immunoassay on either the Roche Modular E170 or Advia Centaur analyzers. On the Roche Modular E170 analyzer, electrochemiluminescent technology is used to measure iPTH level, on which a biotinylated monoclonal antibody and a monoclonal antibody labelled with ruthenium are applied to bind the N-terminal fragment (1-37) and C-terminal fragment (38-84) respectively. After the addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then washed off, followed by the application of a voltage to the electrode to induce chemiluminescent emission which is measured by a photomultiplier.

The Advia Centaur analyzer uses a similar principle as the Roche Modular E170 analyzer. However, instead of using electrochemiluminescent detection technology, the Advia Centaur analyzer uses chemiluminometric detection technology on which dispensing of both acid reagent and base reagent induces the chemiluminescent emission. The Advia Centaur analyzer uses a polyclonal goat anti-human PTH antibody labelled with an acridium ester and a biotinylated polyclonal goat anti-human PTH antibody to bind the N-terminal (1-34) and the C-terminal (39-84) respectively.

The objective of this study was to compare the reliability of iPTH assays between the Roche Modular E170 and the Advia Centaur analyzers, and to determine whether both analyzers can be used interchangeably.

Methods

K3-EDTA plasma samples were collected over the period of four weeks from 24 different patients reported by Canterbury Health Laboratory (CHL) Christchurch to have iPTH levels between 1.7 and 135.0 pmol/L. The whole blood samples were centrifuged at 3700 rpm for seven minutes, plasma separated, and stored at -20°C in plastic Kahn tubes. On the day of analysis, the samples were thawed and mixed using a vortex mixer then centrifuged 3700 rpm for seven minutes prior to analysis.

Calibrations and quality controls were analyzed for both analysers according to the manufacturers’ instructions. The Roche Modular E170 was calibrated using Elecsys PTH CalSet (0.005 pmol/L and 477 pmol/L), while the Advia Centaur was calibrated using intact PTH (iPTH) calibrator (2.68 pmol/L and 88.30 pmol/L). The quality control materials used were PreciControl Bone 1, 2, and 3 for the Roche Modular E170, and Ligand plus 1, 2, and 3 for the Advia Centaur. The patients K3-EDTA plasma samples were assayed in duplicate on both analysers. The coefficient of variance (CV) was calculated from the paired duplicate data. The correlation coefficient and regression analysis was used to determine the association of the results measured by two different analysers. The difference or bias between two analysers was evaluated by plotting the Bland and Altman difference graph and by performing 95% confidence interval (p<0.05) paired t-test using n-1 degree of freedom.

Results

The paired duplicate data from the Roche Modular E170 and the Advia Centaur analyzers gave the internal reliability (CV) of 7% and 3%, respectively. The correlation between the two analysers resulted from both analyzers to identify whether they provided interchangeable results.
The significant difference in results obtained using both analysers may be explained by the fact that both analysers use different calibrator materials thereby producing differences in the expected values of iPTH. This is reflected in the reference ranges of the two analysers where the Roche Modular E170 analyzer reference range was 1.6-6.9 pmol/L while on the Advia Centaur, the reference range was 1.18-8.43 pmol/L.

The difference in these results between both analysers may be caused by two different features found in both assays. First, unlike the Roche Modular E170 that uses electrochemiluminescent technology, the Advia Centaur uses direct chemiluminometric technology and second, the Roche Modular E170 uses monoclonal antibodies while the Advia Centaur uses polyclonal antibodies. A previous study on iPTH assays by Santini et al (2004) indicated that the use of more standardized method of calibration and antibodies that recognize only the biologically active PTH molecule may decrease the wide gap between results obtained from a range of different analysers (4).

This study was limited by a small number of samples as well as low sample volume as the Advia Centaur required a 200µL sample volume while the Roche Modular E170 required only 50 µL of sample. This limited the opportunity to undertake further evaluation such as a sensitivity analysis and a wiser range of iPTH values.

In conclusion, iPTH assays on either analyzer took approximately 18 minutes per sample and assay costs were comparable. However, despite a good correlation, the average difference of iPTH assay between the Roche Modular E170 and the Advia Centaur is significantly different from zero. Therefore, they should not be used interchangeably. Comparison of the two analysers showed that the Advia Centaur had better reliability. Despite a higher plasma volume requirement, the Advia Centaur can be the analyser of choice for iPTH assay in the clinical biochemistry laboratory.

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References

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