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Serum parathyroid hormone concentrations measured by chemiluminescence and electrochemiluminescence methods — are the results comparable in haemodialysis patients with chronic kidney disease?

Stężenia parathormonu w surowicy oznaczane metodą chemiluminescencji i elektrochemiluminescencji — czy wyniki są porównywalne u hemodializowanych chorych na przewlekłą chorobę nerek?

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Abstract

Introduction: Secondary hyperparathyroidism (sHPT) is one of the most common abnormalities found in patients with chronic kidney disease (CKD). Measurement of serum PTH concentrations is crucial in diagnosis and treatment of sHPT. Different methods of serum PTH measurement may provide diverse results. This may have a significant impact on the therapeutic approach if under- or over-diagnosis of sHPT occurs. The aim of this study was to compare the results of serum PTH concentrations measured with two commonly used methods — chemiluminescence (CHL) and electrochemiluminescence (ECL).

Material and methods: Seventy-seven haemodialysis patients with CKD were enrolled into the study. Blood samples were collected before haemodialysis, in the middle of the week. In all patients, serum PTH concentrations were measured using two methods: CHL and ECL.

Results: Serum PTH concentration measured with CHL was significantly higher than that assessed with ECL: 455 pg/mL (352–559) pg/mL *vs.* 383 pg/mL (243–523) pg/mL; $p < 0.0001$. Six patients from the studied cohort were treated with cinacalcet. In these patients, the serum PTH concentration was also significantly higher when measured with CHL than with ECL: 755 pg/mL (294–1216) pg/mL and 607 pg/mL (199–1015 pg/mL); $p = 0.027$, respectively). In three cases serum PTH concentration assessed with CHL method exceeded 300 pg/mL, whereas when measured with ECL it was below 300 pg/mL. Lower serum PTH concentrations could give the rationale to lower cinacalcet dose or to stop such treatment.

Conclusions:

1. Serum PTH concentrations in haemodialysis patients with CKD measured by CHL and ECL methods differ significantly.
2. The choice of method for measurement of serum PTH concentration in these patients may have important clinical implications.

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Key words: *parathyroid hormone; secondary hyperparathyroidism; haemodialysis; chemiluminescence; electrochemiluminescence; cinacalcet*

Streszczenie

Wstęp: Wtórna nadczynność przytarczyc jest jednym z najczęstszych następstw przewlekłej choroby nerek (CKD). Pomiar stężenia PTH w surowicy jest niezbędny dla prawidłowego diagnozowania i leczenia wtórnej nadczynności przytarczyc. Poszczególne metody oznaczania stężenia PTH w surowicy mogą dawać zróżnicowane wyniki, co może mieć istotny wpływ na decyzje terapeutyczne, jak zbyt szybkie lub zbyt późne rozpoznanie choroby. Celem pracy było porównanie stężeń PTH w surowicy oznaczanych przy pomocy dwóch powszechnie wykorzystywanych metod — chemiluminescencji (CHL) i elektrochemiluminescencji (ECL).

Materiał i metody: Badaniem objęto 77 hemodializowanych chorych z CKD. Krew do badań pobierano przed zabiegiem hemodializy, w środku tygodnia. Stężenie PTH w surowicy oznaczono w tej samej próbce metodą CHL i ECL.

Wyniki: Stężenia PTH w surowicy oznaczane przy użyciu CHL były znamiennie wyższe niż, uzyskane przy pomocy ECL: 455 pg/ml (352–559) pg/ml wobec 383 (243–523) pg/ml; $p < 0,0001$. Sześciu chorych leczono cynakalcetem. U tych chorych stężenia PTH w surowicy oznaczane przy użyciu CHL były także znamiennie wyższe, niż oznaczane metodą ECL: 755 pg/ml (294–1216) pg/ml i 607 pg/ml (199–1015 pg/ml); $p = 0,027$. U trzech z tych sześciu chorych stężenie PTH w surowicy oznaczane metodą CHL przekraczało 300 pg/ml, podczas gdy oznaczane metodą ECL było niższe niż 300 pg/ml. Niższe stężenie PTH w surowicy mogłoby być podstawą do podjęcia decyzji o obniżeniu dawki lub zaprzestaniu leczenia cynakalcetem.

Wnioski:

1. Stężenia PTH w surowicy u hemodializowanych chorych na CKD oznaczane przy pomocy metody CHL lub ECL różnią się znamiennie.
2. Wybór metody oznaczenia stężenia PTH w surowicy u hemodializowanych chorych z CKD może mieć istotne implikacje kliniczne.

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Słowa kluczowe: *parathormon; wtórna nadczynność przytarczyc; hemodializy; chemiluminescencja; elektrochemiluminescencja; cynakalcet*

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Introduction

The parathyroid hormone (PTH) is an 84-amino-acid peptide produced and secreted by the parathyroid glands [1]. Main function of PTH is to increase the concentration of serum calcium, mainly acting via parathyroid hormone 1 receptor, which is present in bone and kidney, thus interfering greatly with the calcium–phosphate (Ca–P) balance. The half-life of the PTH molecule is roughly 2 to 4 minutes [2]. As this time circulating PTH is broken down into its peptide fragments, each of a different length and biological activity [3].

Secondary hyperparathyroidism (SHPT) is an abnormal condition commonly observed in CKD patients [4]. Elevated serum PTH and its peptide fragments concentrations adversely affect bone metabolism. Clinical symptoms accompanying the impaired calcium-phosphorus balance, such as abnormalities in imaging tests and renal osteodystrophy, are known as chronic kidney disease — mineral and bone disorders (CKD–MBD) [5]. The clinical importance of developing CKD–MBD is increased cardiovascular and overall mortality caused mainly by vascular calcification [6].

In patients with CKD–MBD, elevated serum PTH concentration reflects the disease's severity, and it is used to determine the type of bone abnormalities, differentiating adynamic and high turnover bone disease [7]. With regard to adynamic bone disease, it is essential to keep PTH concentrations within recommended ranges as an inappropriately low serum PTH concentration intensifies bone damage resulting in pathological fractures and chronic bone pains, significantly lowering patients' quality of life.

In a large cohort of haemodialysed patients with chronic kidney disease, serum PTH concentration fell within the following ranges of serum PTH: 26.3 % *vs.* 34.3 % *vs.* 25.8 % *vs.* 13.7 % for PTH < 150, 150–300, 300–600 and > 600 pg/mL, respectively [8].

Facing this problem in 2009 the Kidney Disease Improving Global Outcomes (KDIGO) guidelines concerning Chronic Kidney Disease-Mineral and Bone Disorder (CKD–MBD), which include the target serum PTH concentration in haemodialysis patients, have been introduced [9]. Some clinical decisions are based on serum PTH concentration. Thus, in order to adjust treatment options, methods used for PTH measurement have to be closely verified in terms of accuracy and repeatability.

Serum concentration of several different forms of PTH can be measured: intact PTH, N — terminal PTH, mid-molecule PTH, and C — terminal PTH. Nowadays, so-called "intact" PTH assays are in common use, based on the ELISA double-sandwich method. Although "intact" PTH assays are widely spread, there has been numerous evidence of their significant inter-test variability. The antibodies used in this generation of assays were originally meant to recognise the C-terminal region and the N-terminal region. Soon afterwards it was confirmed that those antibodies also bind other fragments of the PTH molecule, which are mostly inactive, but some may have inhibitory properties [10, 11].

The aim of this study was to compare the values of serum PTH concentrations in haemodialysis patients with chronic kidney disease, estimated with the use of electrochemiluminescence method and chemiluminescence method, and to estimate the correction factor if inter-method variability occurs.

Material and methods

Seventy-seven haemodialysis patients with chronic kidney disease, treated in the Department of Nephrology, Transplantation and Internal Medicine, Medical University of Silesia in Katowice, were enrolled in the study. The mean age of the patients was 53 ± 17 years. Forty-eight patients were male and 29 were female.

All patients were treated with calcium-based phosphate binders, 20 patients with active vitamin D analogues, and six with cinacalcet.

Blood samples were collected before the haemodialysis session in the middle of the week. The samples were allowed to clot, immediately after that they were centrifuged and divided into three test tubes (2 mL of serum in each). Then the serum was frozen at –40° until assessment.

Serum PTH concentrations were assessed in each sample twice using two methods: ARCHITECT Intact PTH, Abbott, Illinois, USA (CHL) and Elecsys, Roche, Mannheim, Germany (ECL). Both methods are second-generation assays, i.e. "intact" PTH assays. These assays are ELISA-based techniques and use the phenomenon of luminescence. Each of these methods use different antibodies. Serum calcium and phosphate concentrations were were assessed with the University Hospital's Central Laboratory standard methods (using a Beckman-Coulter UniCel DxC 600 analyser).

Shapiro-Wilk test was used to test the variables distribution; Wilcoxon matched pairs test and Mann-Whitney U test were used to evaluate the differences between the variables. Results are shown as a means with 95% confidence index, and differences were considered significant when $p < 0.05$. For each patient the CHL/ECL ratio was also calculated.

Results

In the study population mean serum PTH concentration assessed with CHL method was 455 pg/mL (352–559

pg/mL). This was significantly higher ($p < 0.0001$) than the mean serum PTH concentration assessed with ECL: 383 pg/mL (243–523 pg/mL). The highest serum PTH concentration obtained with CHL was 1659 pg/mL. In this patient the serum PTH assessed with ECL was only 1010 pg/mL. The highest serum PTH concentration using ECL was 4920 pg/mL. In this patient the serum PTH assessed with CHL was > 2500 pg/mL (above the upper limit for CHL without further dilution) and that patient was excluded from the study. The lowest serum PTH concentration in the group was 3.8 pg/mL (assessed with CHL), while in the same patient the serum PTH concentration was 10 pg/mL when using the ECL method (Fig. 1). Mean serum calcium and phosphate concentrations in the study population were 2.09 (2.00–2.18 mmol/L) and 1.95 (1.82–2.09 mmol/L), respectively.

For six patients treated with cinacalcet the results were analysed separately. In three patients receiving cinacalcet serum PTH concentrations were higher than 800 pg/mL for both methods. In two patients treated with cinacalcet there were high discrepancies in the results of serum PTH concentrations: the first patient 361 pg/mL with CHL and 232 pg/mL with ECL; and the second one: 441 pg/mL and 287 pg/mL with the examined methods, respectively.

Considering the large differences in serum PTH concentration using these two commonly used assays, we tried to establish a convenient formula for conversion of the results from one assay to the other, thus the CHL/ECL ratio was calculated. The mean CHL/ECL ratio was 1.34 (1.27–1.41) (Fig. 2).

In order to establish whether this ratio is equally accurate in patients with high and low serum PTH concentrations all patients enrolled into this study were divided into two subgroups by the median value of serum PTH concentration assessed by ECL (233 pg/mL). The mean CHL/ECL ratio in those sub groups was 1.40 (1.34–1.47) for serum PTH below 233 pg/mL and 1.34 (1.26–1.41) for serum PTH above 233 pg/mL. The difference was not significant ($p = 0.056$).

Discussion

In the current study we have found a significant difference in serum PTH concentration in haemodialysis patients with chronic kidney disease, depending on the method of measurement (CHL *vs.* ECL). The differences in PTH concentration using these two commonly used methods reached 34%.

Each of the methods used for serum parathormone (PTH) concentration assessment in our study is based on different antibodies.

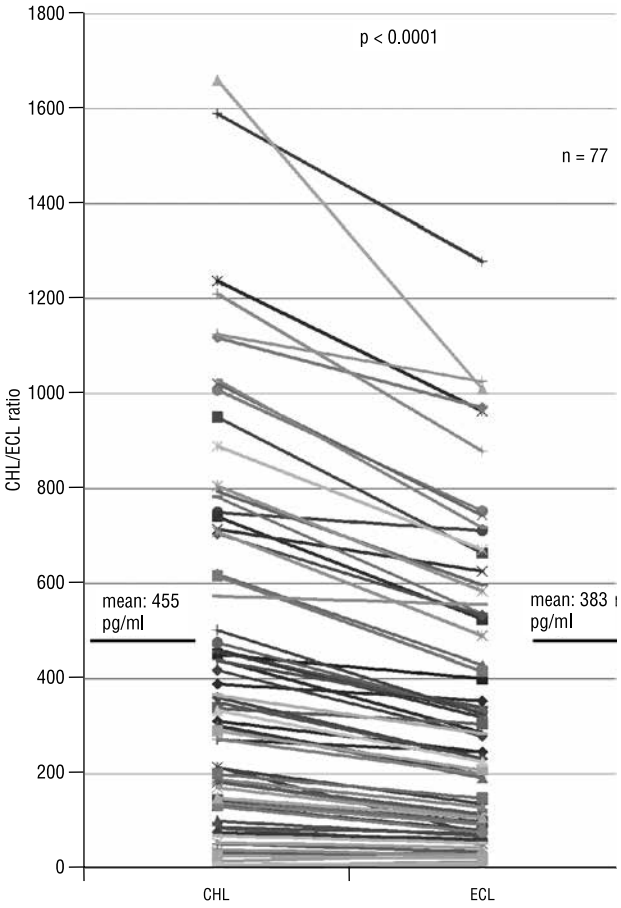

Figure 1. *Serum PTH concentrations in 77 haemodialysis patients with CKD*

Rycina 1. *Stężenia PTH w surowicy u 77 hemodializowanych chorych na CKD; PTH — parathyroid hormone, CHL — PTH concentrations measured with chemiluminescence method, ECL — PTH concentrations measured with electrochemiluminescence method*

ARCHITECT (Abott) is a chemiluminescent microparticle immunoassay (CMIA in short: CHL). It uses acridinium ester as a tracer, which causes lightening in the presence of hydrogen peroxide. This method consists of combining intact PTH (iPTH) present in the serum sample to anti-PTH antibodies in order to create antigen-antibody complexes. Afterwards a secondary antibody is added in order to detect these complexes by triggering a chemiluminescent reaction. Specifications of the measured fragments of PTH and antibodies that are used in this method are not given by the manufacturer of this test [12].

ELESCYS assay (Roche) is based on electrochemiluminescence (ECL) — a process of emitting light as a result of electrochemical reactions. Two types of antibodies are used: N-terminal antibody and one labelled with ruthenium complex C-terminal. These antibodies are directed toward 26–32 PTH and 37–42 PTH [13].

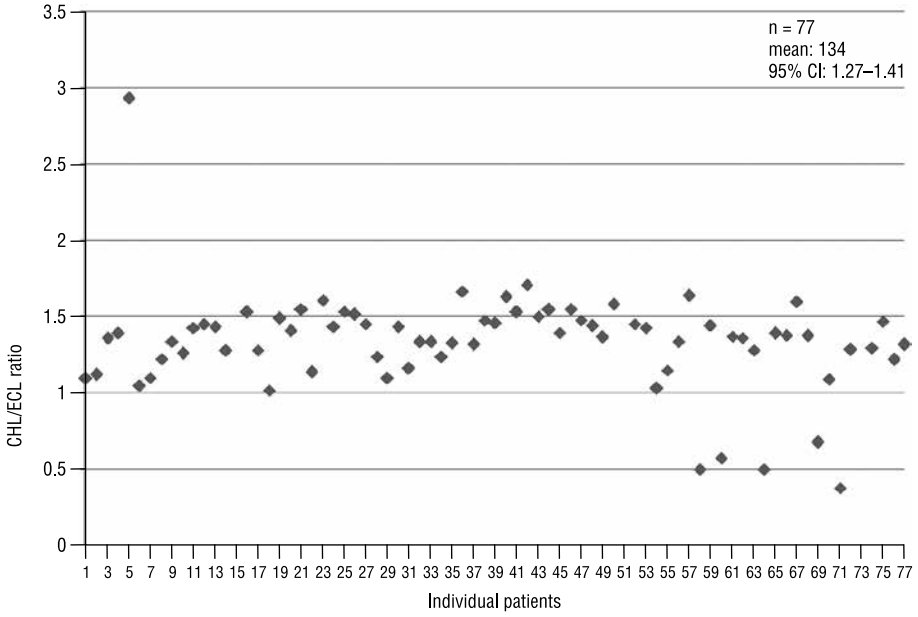

Figure 2. *CHL/ECL ratio for PTH concentrations*
Rycina 2. *Wskaźnik CHL/ECL dla stężeń PTH w surowicy*

In our study we observed that the results of serum PTH concentration obtained with the CHL method were higher than those using the ECL method. Results from recent studies have also reported considerable variability in PTH serum concentrations. In a study by Monge et al. [14] the authors showed that CHL results [238 (140–434) pg/mL] were significantly ($p < 0.001$) higher than the results obtained with the ECL method [182 (109–338) pg/mL]. This is in agreement with the results of our current study.

Having the results of PTH concentrations measured by both ECL and CHL methods, we were able to calculate the CHL/ECL ratio. The mean value of this ratio was 1.34, and it was very similar to the value obtained by Monge et al., who calculated the ratio to be 1.3.

There are many potential causes of these inter-method differences. In serum samples, many forms of PTH can be found, like C-terminal and N-terminal fragments, amino-PTH, and many others. Another potential cause of these discrepancies in serum PTH concentrations may be related to the fact that these two methods differ according to the fragments of PTH they are measuring. Moreover, there may be mistakes caused by other proteins with similar epitopes, or heterophilic antibodies in serum (like in patients who were exposed to animals or animal serum products) [12]. Lack of standardisation of methods measuring serum PTH concentrations may be another reason for the inter-method discrepancies. In 1981 the only reference preparation for these methods was made with purified human PTH. The majority of commonly used methods are calibrated against synthetic 1–84 PTH, but recently there have been no attempts to standardise these methods [15].

In our study six patients were treated with cinacalcet. It is known that cinacalcet increases the sensitivity of calcium receptor to the serum calcium and reduces serum parathormone (PTH) concentration [16, 17]. After measuring serum PTH concentration using both of the abovementioned methods, it turned out that, according to ECL assay, in two of these patients the dose of cinacalcet could have been reduced or cinacalcet treatment could have been ceased.

These discrepancies are of major importance because usually there is no information on the result sheet obtained from commercial laboratories concerning the method used for serum PTH concentration assessment.

Our study has some limitations. The most important one is the rather small study population. Nevertheless, even in such a small group of patients we managed to find a significant difference between CHL and ECL methods of serum PTH concentration assessment.

Conclusions

Based on the results obtained in the study, we can conclude that:

1. Serum PTH concentrations in haemodialysis patients with chronic kidney disease measured with CHL an ECL methods differ significantly.
2. The choice of method for the assessment of serum PTH concentration these patients may have important clinical implications.

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