TARGET RANGES AND PTH PROFILE IN A WEST AFRICAN POPULATION USING 2ND AND 3RD GENERATION TESTS

Abstract

Introduction: Bone metabolism disorders are one of the most frequent and serious complications of kidney disease, and are associated with high morbidity and mortality. In order to prevent these bone complications, the Kidney Disease Improving Global Outcomes (KDIGO) expert committee recommends maintaining PTH values within a target range dependent on the race of the population and the generation of test used. The aim of this study was to propose target intervals for monitoring PTH in Ivorian haemodialysis patients according to test generation, and to establish the PTH profile of these patients.

Material and methods: This was an analytical cross-sectional study involving 86 black African subjects in apparent good health for the determination of PTH target intervals and 200 black African haemodialysis patients for the PTH profile. Target ranges were determined simultaneously on a 2nd generation Maglumi 800and 3rd generation kitVidas (Whole PTH) according to KDIGO (2009) recommendations. The PTH profile of hemodialysis patients was established in comparison with the pre-established target ranges.

Results: The target PTH ranges for dialysis patients were 280.4 to 1261.8 pg/ml for the Maglumi kit® and 83.8 to 377.1 pg/ml for the Vidas kit®. The mean PTH value in healthy subjects was 21.99 pg/ml, compared with 473.3 pg/ml in haemodialysis patients. According to the target interval established on the Vidas® kit, 34% of dialysis patients had a PTH value above the upper limit of normality and were therefore at risk of bone complications.

Conclusion: The PTH values of black African Ivorian hemodialysis patients according to the pre-established target intervals were significantly higher in our population than those of Caucasian and Asian populations described in the literature.

Keywords: PTH, West African, target range

1- Introduction

Chronic kidney disease (CKD) is characterized by the persistence for more than three months of a decrease in glomerular filtration rate (GFR) below 60ml/min/1.73m2 associated or not with proteinuria[1,2]. The course of the disease is fraught with complications, in particular mineral and bone metabolism disorders (BMD), which can lead to falls, fractures and vascular calcifications. These BMDs, resulting from secondary hyperparathyroidism, are a frequent and serious complication of the disease and are associated with high morbidity and mortality [3,4].

With the aim of preventing these bone complications, the kidney disease improving global outcomes (KDIGO) expert committee recommends biannual or annual parathyroid hormone dosing in chronic kidney disease patients on dialysis, as well as maintaining PTH values within a target range corresponding to 2 and 9 times the upper normal limit [5]. However, the interpretation of PTH values in dialysis patients is subject to many variations, including the race of the population and the generation of test used. The current PTH assay methods are 2ndand 3rd generation(Whole PTH)[6,7]. Although 2nd generation assays have the disadvantage of assaying other fragments in addition to intact PTH (the active part of PTH), which are more dependent on regional and ethnic population variability, they are still available and widely used in our resource-limited African context. The objectives of this study were to propose target PTH monitoring intervals for Ivorian haemodialysis patients, based on the generation of tests, and to establish the PTH profile of these patients.

2- Materials and methods

2.1- Conception of Study

This was a cross-sectional analytical study, initiated by the Biochemistry Department of Pharmaceutical and Biological Sciences at the Université Félix HouphouëtBoignyd'Abidjan, in collaboration with the urgent medical help service (SAMU), the national blood transfusion center (CNTS) in Abidjan and the national

center of prevention treatment ofrenal failure (CNPTIR). It has been approved by the National Ethics and Research Committee (CNER) of the Ministry of Health and Public Hygiene of the Republic of Côte d'Ivoire under number N°138-22/MSHP/CNESVS-km. Each participant who gave informed consent to take part in the study signed an individual consent form.

The study included 86 subjects in apparent good health for the determination of target intervals and 200 haemodialysis patients for the PTH profile.

Apparent good health was defined according to the CNTS clinico -biological criteria for blood donation . Clinically, the state of good health was assessed by a medical visit to the said center during which blood pressure was taken. Subjects declared 'unfit' for apparent chronic or acute pathologies were not included in the study. Elderly subjects under 18 years of age and pregnant women were also excluded. At the biological level, in addition to negative serology for HIV, syphilis, hepatitis B and C, selection was mainly concerned with normal blood calcium, phosphorus and 25-OH vitamin D concentration thus eliminating factors that could influence PTH concentrations, according to values previously established in the presumably healthy Ivorian[8,9].

Hemodialysis patients were black West African adults treated with hemodialysis at the rate of two four-hour sessions per week, who came for their annual follow-up check-up at the SAMU biology laboratory. Patients with incomplete records were excluded from the study.

2.2- Methods

A survey form and a blood sample were taken from each subject. The survey form was used to collect socio-demographic and clinical data. Venous blood samples were taken in the morning, from the cubital vein, on fasting state using a Vacutainer® system in dry tubes without anticoagulant. Whole blood was centrifuged at 4,000 rpm for 5 minutes. The collected serum was aliquoted, labeled according to a defined coding scheme and stored at -20°c until assay. Ionized calcium, phosphorus and 25OH vitamin D levels were measured on the day of sampling, before aliquots were taken, to exclude all patients with disturbances of these parameters, indicative of dysfunctional phosphocalcic metabolism. Ionized calcemia was measured using an

OPTI CCA-TS2® electrolyte and blood gas analyzer, a microprocessor-based instrument that measures optical fluorescence from discrete sensors called optical electrodes (optodes). Phosphoremia was measured on a HITACHI 704® automatic phosphorimeter using the end-point UV ammonium molybdate photometry method. PTH was measured simultaneously on a 2nd generation kit (SNIBE MAGLUMI 800®) and a 3rd generation kit (VIDAS BIOMERIEUX®) two weeks after sampling. Target intervals on both kits were determined in accordance with KDIGO [10]recommendations, using the following steps:

- The determination of PTH in apparently healthy subjects taken from blood donors in Abidjan who do not show any disturbance of the serum calcium and phosphorus values;
- determination of the normality interval in these healthy subjects using the non-parametric quantile method, by aligning the values obtained in ascending order and eliminating 2.5% of the low values and 2.5% of the high values, i.e. 5% of the values.
- Determination of the dialysis patient's PTH target range by multiplying the value of the upper limit of the normality range by 2 and 9.

The PTH profile of hemodialysis patients was established in comparison with the pre-established target ranges.

2.3- Statistical Analysis

Qualitative data are presented as patient numbers and percentages, while quantitative data are presented as means, standard deviation, median, range (minimum, maximum).

The PTH normality interval was determined using the non-parametric quantile method.

1- Results and Discussion

3.1- General characteristics of the study population

The characteristics of apparently healthy subjects and haemodialysis patients are recorded in Tables 1 and 2 respectively.

Table 1: Characteristics of blood donors

Parameters	Distribution	Numbers	Percentage
Gender	male	65	75.6%
	Female	21	24.4%
Age (years)	[18-30[	29	33.72%
	[30-40[	28	32.56%
	[40-50[	22	25.58%
	≥50	7	8.14

Table 2: General characteristics of hemodialysis patients

Parameters	Distribution	Numbers	Percentage
Gender	Male	131	65.5%
	Female	69	35.5%
Age (years)	< 40	53	26.5%
	[40 – 50[	72	36%
	[50 – 60[	47	23.5%
	≥ 60	28	14%
Age of dialysis (months)	[3-60[	106	53%
	≥ 60	94	47%
Associated pathologies	HTA	186	93%
	diabetes	14	7 %
Calcium and/or vitamin D supplementation	yes	162	81%
	no	38	19%

3.2- Determination of target ranges on both kits

The target range for dialysis PTH on the Maglumi kit is 280.4 to 1261.8 pg/ml (Table 3). The target PTH range for dialysis patients on the Vidas kit is 83.8 to 377.1 pg/ml (Table 4).

Table 3: PTH target range for dialysis patients on the Maglumi 800® kit

Parameters	PTH (pg/ml)
Median (min; max) (healthy subjects)	84.86 (12.34 ; 48.2)
Normality interval (healthy subjects) (Percentile 2,5% - Percentile 7,5%)	30.1 - 140.2*
Target interval of the dialysis	280.4 – 1261.8

*** Upper normal limit (ULN)**

Table 4: PTH target range for dialysis patients on the Vidas® kit

Parameters	PTH (pg/ml)
Median (min; max) (healthy subjects)	21.3 (7.8 ; 43.1)
Normality interval (healthy subjects) (Percentile 2,5% - Percentile 7,5%)	9.6 – 41.9*

Target interval of the dialysis	83.8 – 377.1

3.3- PTH profile using the 3rd generation Vidas kit Biomérieux (iPTH)

The mean PTH value in healthy subjects was 21.99 pg/ml, compared with 473.3 pg/ml in hemodialysis patients (Table 5).

According to the target range established on the Vidaskit, 34% of dialysis patients had a PTH value above the upper limit of normality and were therefore at risk of bone complications (Table 6).

	Mean	Standard deviation	Median	Mini	Max
PTH of healthy subjects	21.99	7.18	21.3	7.8	43.1

Table 5: Characteristics of parathyroid hormone in the study population

(pg/ml)					
PTH of hemodialyse patients (pg/ml)	473.3	493.7	245.3	14.3	1600

Table 6: Distribution of hemodialysis patients by target interval on the Vidas kit.

PTH values	In Target interval (83.8 – 377.1 pg/ml)	higher than the interval (>377.1 pg/ml)
Numbers	132	68
Percentage	66%	34%

4.1- PTH target ranges in our hemodialysis population

The PTH target ranges determined from the respective normality ranges, vary from 280.4-1261.8 pg/ml for the Maglumi kit and 83.8-377.1 pg/ml for the Vidas kit. Thus,

in Ivorian dialysis patients, the accepted PTH threshold values (values above which bone complications are likely to develop) are 1261.8pg/ml and 337.1 pg/ml for the Maglumi 800® and Vidas® kits respectively.

The threshold value obtained in our study from the Maglumi 800® kit is very different from that obtained by Gannagé-yared et al.[11]and Laradi et al. [12]on 2nd generation kits (Diasorin®, Imilitte 2000 DPC-5®) working on Lebanese and French populations. The threshold values on these kits were 994.45pg/ml and 783pg/ml respectively. These differences in threshold values for tests of the same generation can be explained by the difference in race of the populations studied. Several factors are mentioned in this respect:

Concerning 3rd generation assays, the PTH threshold value obtained by Cavalier et al.[13], using the FurjirebioLumipulse G® 3rd generation test from Belgian subjects was similar to that obtained in our study. It thus appears that the specificity of 3rd generation tests gives similar results regardless of patient race.

Similarly, irrespective of race and generation of PTH kit used, a difference was observed between the threshold value established in our study on the Maglumi 800 ® kit and that established by Cavalier et al.[14] in Côte d'Ivoire on a similar population using an identical generation test (Roche elecys). On the other hand, for 3rd generation tests, the value obtained in our study and that obtained on the Furjirebiolumipulse G® test from African subjects are superimposable. This difference observed on a kit of the same generation may be due to the existence of cross-reactions in second-generation assay methods, or to a problem of calibration of the different intact parathyroid hormone assay methods. Indeed, some authors, such as Migliardi and Marranca [15] in a study of parathyroid hormone measurement by two second-generation assays, found that recovery tests performed as a percentage of cross-reactions varied from one method to the other. This could be the reason for the differences observed. The UK National External Quality Assessment Service (UK-NEQAS)[16]shows that there are calibration differences between methods and therefore asserts that differences between PTH values obtained with different kits could be considerably reduced if all methods had similar recovery percentages. This observation was confirmed a few years later by the standardization of 3rd generation PTH tests, for which values remain superposable from one kit to another and from one population to another.

The PTH threshold value obtained in our study using the Maglumi kit is different from that obtained using a 3rd generation kit (FujirebioLumipulse®) in a population of African subjects presumed to be healthy [14]. Indeed, as revealed by several studies, 2nd generation kits are subject to interference by assaying non 1-84 fragments of PTH, leading to an overestimation of PTH concentrations compared with 3rd generation kits, which are clearly more specific [17–19].

4.1- PTH profile in haemodialysis patients

The mean PTH value in haemodialysis patients is much higher than in the healthy population, reflecting hyperparathyroidism secondary to renal failure. On the Vidas® kit, which is much more specific for intact PTH, the target range determined was 83.8 - 377.1 pg/ml. The interpretation of the PTH values of hemodialysis patients according to the target range determined on this kit (3rd generation) shows that 34% of patients had a PTH value above the upper limit of normality, and were therefore at risk of developing bone complications. Furthermore, despite the high PTH levels, the bone complications encountered were mainly minor ones such as bone pain. More severe complications such as fractures and bone deformities were present in only 13% of cases. On the Vidas kit, the mean PTH value was 473.3 pg/ml. This value found in our African population is quite different from the average intact PTH values determined in other populations. Indeed, in a recent study published by Evenepoel et al.[20], the mean values of 3rd generation intact PTH found in Belgian and Japanese populations were 268 pg/ml and 168 pg/ml respectively. Similarly, Chan et al. [21], found a mean value of 496 pg/ml in African-Americans. One possible explanation for these disparities could be differences in responsiveness to PTH in target organs such as bone by these different groups. Indeed, ethnic differences in skeletal response to PTH have been demonstrated, notably in African-American patients compared to white American patients, where for comparable PTH levels, bone remodeling rates were different, lower in black Americans than in white Americans [22]. Similarly, Evenepoel et al. [20]found significantly lower skeletal remodeling in Japanese patients than in Belgian patients, but unexpectedly, skeletal reactivity to PTH was rather lower in Japanese patients than in European patients.

The mechanisms of this difference in bone remodelling activity remain speculative at present. Racial differences in PTH receptor concentration in bone have been suggested. Indeed, the effect of PTH is mediated via the PTH receptor PTHrP, and a lower concentration of these receptors in black subjects than in Caucasians could explain at least part of these results[22]. Similarly, vitamin D, considered a known modulator of PTH action [23,24], has been incriminated in skeletal resistance to PTH in African-Americans due to the adaptive mechanism put in place to compensate for the relative 25-hydroxy vitamin D deficiency frequently observed in African-Americans [25,26]. However, Cosman et al. [27] demonstrated that the role of 25-hydroxy vitamin D in this apparent resistance to PTH is minor at best. Racial differences in the polymorphism of the vitamin D receptor gene have been reported in some studies [28]. This polymorphism therefore needs to be examined in different populations. Finally, it is currently recognized that many local factors and cytokines affect bone remodelling[29,30]. Possible racial differences among these factors await further research.

However, Sawaya et al [22] showed that high PTH levels in African-Americans were not associated with more severe bone disease.

All this suggests that further investigations, particularly into skeletal reactivity to PTH, need to be carried out in other ethnic groups or between different regions of the world to better understand the mechanisms behind these disparities in PTH values. Furthermore, while in Europe and the USA, the KDIGO committee recommends maintaining PTH values at levels between 2 and 9 times the ULN, the Japanese Society recommends a lower, narrower PTH target of around 1 to 4 times the ULN [31]. The treatment targets are therefore completely different. And for these authors, these narrower threshold targets contribute to better management of bone complications in Japanese patients.

Would it therefore make sense to adapt PTH treatment targets according to population type? According to Malluche and Monier-Faugere[32], potential differences are important to take into account when defining target PTH levels in patients with end-stage renal disease.

In black patients, even if the KDIGO recommendations seem appropriate, more in-depth studies including assessment of bone activity (bone remodeling marker, scintigraphy, etc.) in relation to PTH concentrations would enable a more specific target range to be set.

2- Conclusion

For a better interpretation of the values of PTH, the main hormone regulating bone metabolism, we were able to determine PTH target ranges adapted to our population on 2nd (Maglumi 800) and 3rd generation (Vidas) kits, which are 280.4-1261.8 pg/ml for the Maglumi kit and 83.8-377.1 pg/ml for the Vidas kit.

Interpretation of the PTH values of the hemodialysis patients in the study (black Ivorian Africans), based on the pre-established target ranges, showed significantly higher PTH values in our population than in the Caucasian and Asian populations (described in the literature), partly due to differences in PTH reactivity in the target organs by these different populations. Therefore, even if the KDIGO recommendations seem appropriate for blacks, more in-depth studies including assessment of bone activity (bone remodeling marker, scintigraphy) in relation to PTH concentrations would enable a more specific target range to be set.

3- Limitations of our study

The confrontation of high PTH values with bone remodeling markers and scintigraphy would have made it possible to better study bone complications.

4- Ethical approval and consent

This study was approved by the Comité National d'Ethique et de la Recherche (CNER) of the Ministry of Health and Public Hygiene of the Republic of Côte d 'Ivoire under number 138-22 /MSHP/CNESVS-km. A free and informed consent form was obtained from all participants.

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