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STUDY OF VARIABILITY OF SERIAL PARATHORMONE ON PATIENTS DIALIZED

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Key words: *phosphocalcal homeostasis, serum Ca, P, PTH, dialyzed patients*

INTRODUCTION

Bone mineral disease is a frequent complication of chronic kidney disease and comprises a broad spectrum of mineral metabolic disturbances that occur in this clinical context and have both bone and extra-sciatic consequences, defined by one or more of the following: anomalies of serum Ca, P, PTH or Vitamin D, abnormalities in turnover, mineralization, volume, linear growth or bone hardness and vascular calcifications or soft tissues.

Parathyroid hormone stimulates calcium and phosphate from the bones, increases calcium absorption in the intestine and renal excretion of phosphate. The secretion of parathyroid hormone and release into the bloodstream is accomplished by exocytosis.

The kidney and liver rapidly remove parathyroid hormone from the circulation. The clinical significance of parathyroid hormone is to investigate altered phospho-calcic homeostasis and to monitor renal dialysis. Beginning with stage 3 of chronic kidney disease (CKD), the ability of the kidneys to properly excrete the phosphate decreases, causing hyperphosphatemia and secondary hyperparathyroidism, while reducing intestinal calcium absorption, hypocalcaemia and PTH growth. In chronic kidney disease, the kidney fails to respond adequately to the growth of parathyroid hormone, which normally stimulates phosphaturia and reduces calcium.

Chronic kidney disease (CKD) has a growing prevalence in both Romania and the world. Currently, 1 in 10 Romanians are suspected of having this disease, and almost 10,000 have renal replacement therapy (TSFR), the number being rising.

MATERIAL AND METHODS

Chronic kidney disease evolves into chronic renal failure through the slow, progressive destruction of nephrons. Among the manifestations associated with chronic renal failure are included phospho-calcic metabolism disorders. This is accentuated as the glomerular filtration rate is increased.

The biochemical parameters of phospho-calcic/bone metabolism should be measured periodically, dynamically, in all patients with proven renal dysfunction. The most useful parameters are calcemia, phosphathemia and parathormone (PTH). Frequency of monitoring the biochemical parameters of bone mineral metabolism depends on the stage of chronic kidney disease and clinical condition.

For the purpose of this study, 87 dialysed patients aged 21 to 88 years who were admitted to the Department of Nephrology and Hemodialysis of the Bacau Emergency County Hospital during the year 2016 with various severe kidney diagnoses, most with chronic renal disease or chronic renal failure in various stages.

The serum levels of parathormone, calcium and phosphorus were determined in these patients.

For the determination of calcium and phosphorus, the COBAS 6000 analyzer, manufactured by Roche-HITACHI, Germany (Figure 1), was used. Determinations were made on patient sera obtained by centrifugation of the blood collected in anticoagulant tubes. Centrifugation was performed for 5 minutes at 3000 rpm using the ROTOFIX 32 A centrifuge, manufactured by Hettich Zentrifugen, Germany (Figure 2). .

Determination of calcium

Stability of the sample - the centrifuge separated serum is stable for 7 days at 15-25 ° C; 3 weeks at 2-8 ° C; 8 months at (-15) - (-25) ºC.

The principle of the method - the ionic calcium reacts with the o-crezophthalein complex (o-CPC), forming a violet-colored complex in the presence of alkaline phosphatase. By adding 8-hydroxyquinoline, iron and magnesium interference is prevented. The color intensity of the complex formed is measured photometrically and is directly proportional to the calcium concentration.

Reagents used for determining calcium (Figure. 3) are stable until the expiration date, before opening, if stored at 2-8 ° C, and on the analyzer board the stability of the cassette is 3 weeks.

Reference values - vary by age.

Determination of phosphorus

Stability of the sample - the centrifuge separated serum is stable 24 hours at 15-25 ° C, 4 days at 2-8 ° C; 1 year at (-15) - (-25) ºC.

The principle of the method - by reacting inorganic phosphorus with ammonium molybdate, in the presence of sulfuric acid, forms an ammonium phosphomolybdate complex. The concentration of phosphomolybdate obtained is directly proportional to the concentration of inorganic phosphorus and the color intensity is measured spectrophotometrically.

Reagents used for phosphorus determination (Figure 4) are stable up to the expiration date before opening if stored at 2-8 ° C and on the analyzer board the stability of the cassette is 12 weeks.

Reference values - differ by gender and age.

Determination of parathyroid hormone

For the determination of parathormone (PTH), the COBAS E 411 analyzer, produced by Roche-HITACHI, Germany (Figure 5)

The PTH determination method used in the medical analysis laboratory of Bacau County Emergency Hospital is a third-generation method, an ECMC immuno-chemical detection technique; intact PTH is determined, thereby eliminating the interference exerted by the metabolites; the two monoclonal antibodies used are reacted with epitopes corresponding to amino acid regions 26-32 and 37-42 of the PTH molecule.

Sample stability - Separated centrifuge serum is stable for 8 hours at 15-25 ° C; 2 days at 2-8 ° C; 6 months at (-15) - (-25) ºC.

The principle of the method - is the sandwich principle with 2 incubations: - at the first incubation, the serum and the specific monoclonal PTH antibodies bind to a complex deruten and form a sandwich complex, - at the second incubation, the complex formed binds to the solid phase and interacts the cubiotin and streptavidin. Figure 6 shows the reagent box used for PTH determination. Reagents are set up to expiration date before opening and stored at 2-8 ° C, and analyzer benchmark stability is 56 days.

Reference values - 15-65 pg/mL

Fig. 1. COBAS 6000 Analyzer

Fig. 2. ROTOFIX 32 A2 centrifuge

Fig. 3. The reagent box for calcium determination

Fig. 4. Phosphorus Reagent Reagent Box

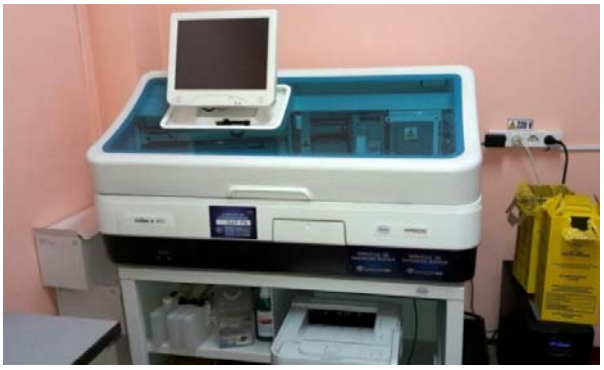

Fig. 5. COBAS E 411 Analyzer

Fig. 6. Reagent Box for PTH determination

Preparing the patient

- blood collection is obligatory for à jeun;
- venous blood will be harvested in the vacutainer without anticoagulant with / without separating gel;
- causes of sample rejection - haemolysis specimen.

a) For the determination of calcium

- Do not take calcium supplements 8-12 hours before; it is not possible to determine the calcium in patients treated with EDTA or who have received radiological contrast agents.
- the harvest is done in the morning (because there are diurnal variations), in the clinostatism (there are variations in posture, because half of the calcium is linked to the protein, in the hypostasis, calcium and proteinemia are lower than in orthostatism). At harvest, venous stasis with the tourniquet will be avoided because it induces false values. If the use of the tourniquet is indispensable, the sample will be taken more than 1 minute after the restoration of the movement. At harvesting and then during sample handling, do not use powdered calcium carbonate gloves.

b) *For the determination of phosphorus*

- postprandial, the serum phosphate level is increased;
- normally, in children, phosphorus levels are increased;

There are seasonal variations in phosphorus concentration (minimum in the winter months, maximum in May-June) and circadian (minimum evening and maximum in the morning);

- Stress increases serum phosphorus levels.

c) *For determination of parathyroid hormone*

- there is a diurnal variation in the level of PTH in the blood, therefore harvesting for PTH is done in the morning when its level is minimal;
- PTH plasma concentration can only be interpreted if the laboratory technique used is known.

RESULTS AND DISCUSSIONS

In any disease, the objective of biological exploration is to detect the degree of suffering of the affected organ, and to evaluate functional consequences on the whole organism or organs.

Data obtained from laboratory tests, along with clinical and paraclinical data, allow a differential diagnosis, confirming or refusing clinical diagnosis or allowing the disease to be detected at a rather early stage of evolution.

Laboratory investigations are individualized and periodically repeated, thus becoming a guiding criterion in assessing the disease-eradication goal.

Statistics show that urinary tract disorders are not uncommon, so that 40% of women and 15% of men do at least one urinary infection during their lifetime. Also, statistics show that 1 adult in 10 has chronic kidney disease, which means that about 2 million people are affected. Of these, more than 8,000 are treated by dialysis or kidney transplantation.

In the 87 patients, calcium, phosphorus, and parathyroid hormone levels were determined in different phases of the substitution treatment.

The distribution by age group of the investigated patients shows that the highest share is between 71-80 years old and the lowest proportion of patients aged 20-30 years (Figure 7).

The gender distribution of the patients investigated during 2016 shows that 46 patients (52.87%) were women and 41 (47.13%) were men, the incidence of the disease is approximately equal to the two sexes (Figure 8).

Concerning the distribution according to the patient's environment, the share of the disease in the urban environment is slightly higher than in the rural area (Figure 9).

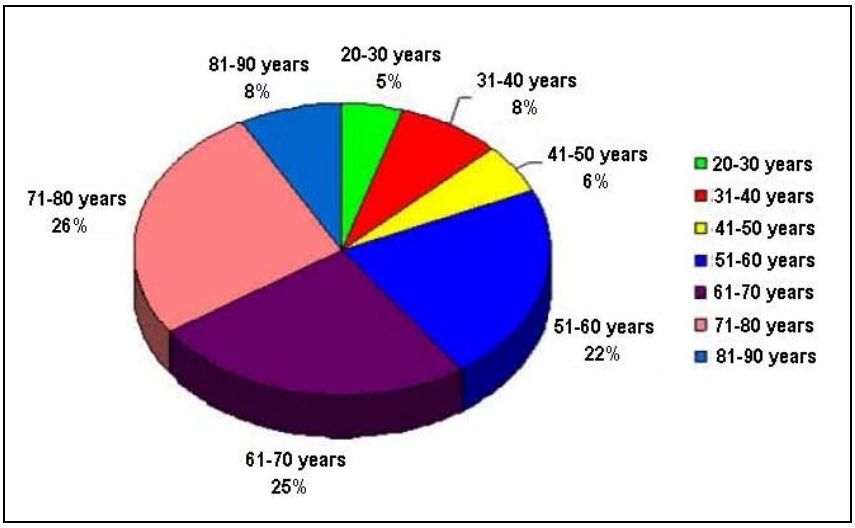

Fig. 7. Distribution by age group of investigated patients

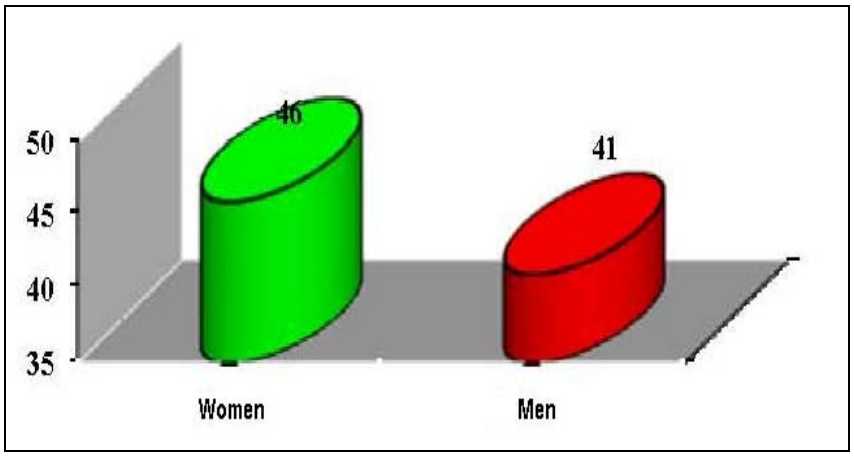

Fig. 8. Sex distribution of the investigated patients

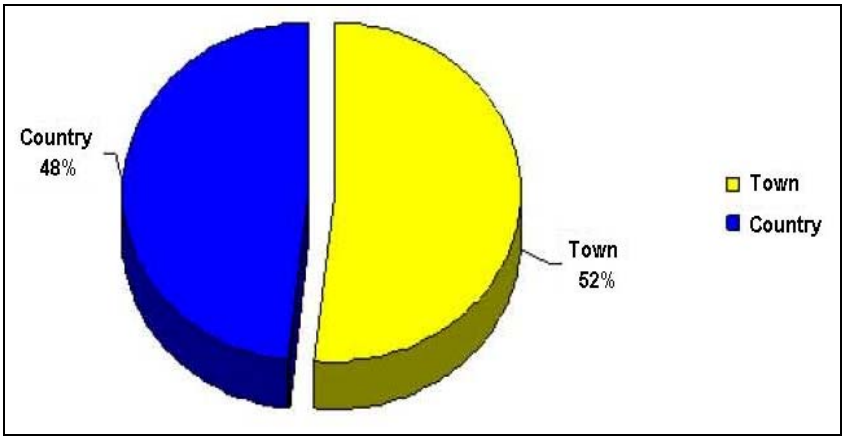

Fig. 9. Distribution of patients by place of origin

The graphical representation and the value variation of the three parameters in the patients analyzed are shown in figures 10, 11 and 12.

PTH reference values are (15-65) pg / l.

The values below the lower limit of the reference range were obtained in 3 patients, that is 3.45% of the total investigated patients. Normal values were shown in 17 patients, that is 19.54% of the total, while the highest share was seen in patients with elevated PTH, 67 patients, respectively 77.01% of the total (Figure 10). It was also found that the patient with the highest phosphorus value also has the highest value of PTH and the lowest value of calcium (Figures 10, 11). From the data obtained, there is a very high correlation between the values of PTH and phosphorus values, and between these two parameters there is a close interdependence.

In patients with chronic renal failure, calcium depletion occurs at a slower rate than phosphorus elevation, and PTH tends to stop this increase in phosphorus. Normally, PTH is a hormone that stimulates phosphaturia and reduces calcium. But in chronic renal disease, the more advanced the stage, the better the ability of the kidneys to properly excrete the phosphate, causing hyperphosphatemia, hypocalcemia, and PTH growth (Figure 13).

Calcium reference values are 8.2-10.4 mg/ dl.

Only one patient in the investigated group had a very low calcium value, namely 4.79 mg/dl (fig.11).Values ranging from (6-8.2) mg/dl to 18 patients, that is 20.69% of all investigated patients. Values above the upper limit of the reference range were 5 patients, namely 5.74%, with the highest proportion of the investigated patients having values that are within the normal range.

The phosphorus reference values are 2.7-4.5 mg/dl.

Phosphorus values ranging from (2-2.7) mg/dl to 3 patients, that is 3.45% of the total investigated patients. Values above the upper limit of the reference range showed 40 patients, that is 45.98%, while 44 patients had normal values, namely 50.57% of the total (Figure 12).

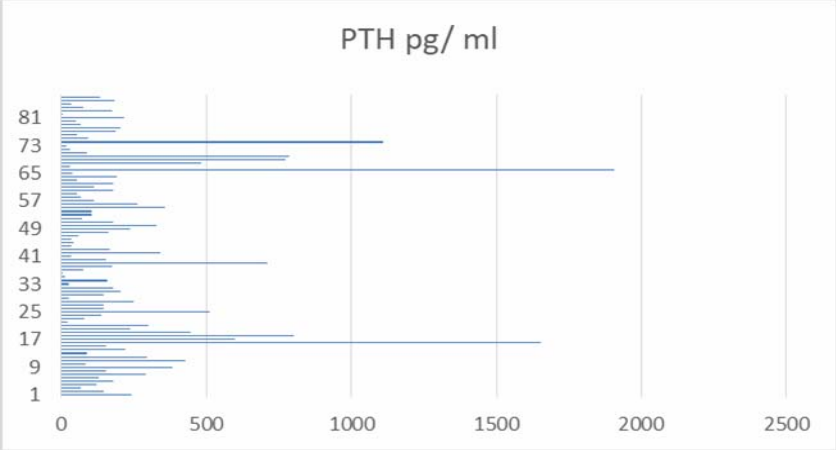

Fig. 10. Variation of PTH values in dialysis patients

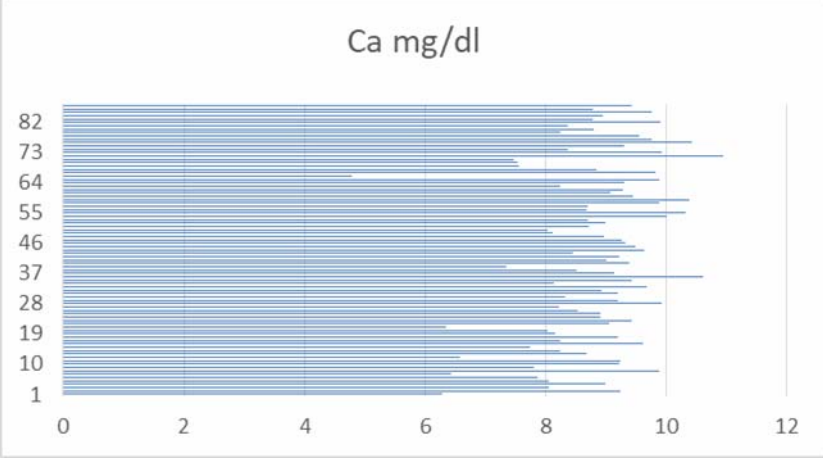

Fig. 11. Variation of calcium in dialysis patients

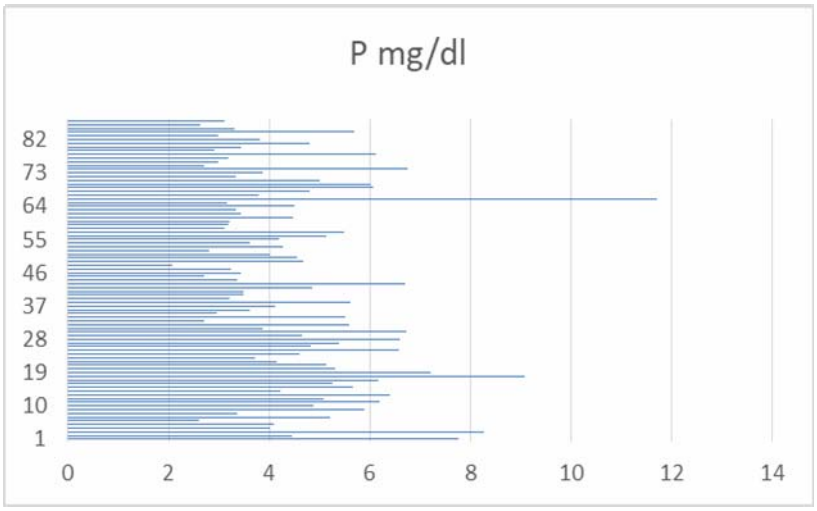

Fig. 12. Variation of phosphorus values in dialysis patients

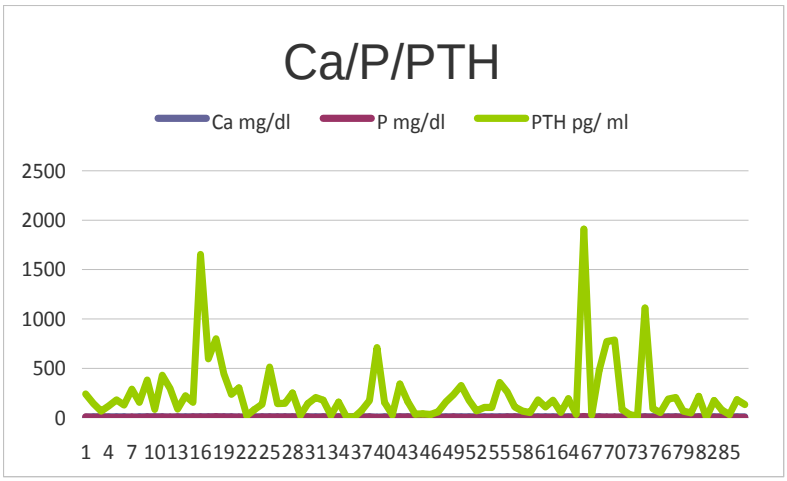

Fig. 13. Variation of the three parameters in BCR (hyperphosphatemia, hypocalcaemia and increase in PTH) in dialysis patients

CONCLUSIONS

From the analysis of the data obtained for the 87 investigated patients, the following were found:

Only one patient in the investigated group had a very low calcium value of 4.79 mg/dl;

It has also been found that the patient with the highest phosphorus value has the highest value of PTH and the lowest value of calcium.

From the data obtained, there is a very high correlation between the values of PTH and the values of phosphorus, between these two parameters there is a close interdependence.

In patients with chronic renal failure, calcium depletion occurs at a slower pace than phosphorus elevation, and PTH tends to stop this phosphorus build-up.

Normally, PTH is a hormone that stimulates phosphaturia and reduces urinary calcium. But in chronic kidney disease, the more advanced the stage, the better the ability of the kidneys to properly excrete the phosphate, causing hyperphosphatemia, hypocalcaemia and PTH growth.

ABSTRACT

87 dialysis patients aged between 21 and 88 years who were admitted to the Nephrology and Hemodialysis Section of the Bacau Emergency County Hospital during the year 2016 with various severe kidney diagnoses were investigated, most of whom had chronic renal disease or chronic renal failure in various stages. The serum levels of parathormone, calcium and phosphorus were determined in these patients. From the data obtained, there is a very high correlation between the values of PTH and phosphorus values, between these two parameters there is a close interdependence. In

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