Experimental Research on Variation in Serum Calcium, Phosphorus, Sodium, Potassium and the ESR during Bone Transplant

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The aim of this study was to analyze the plasma concentration of chemical elements in blood after bone transplantation, which, correlated with clinical picture, can give us some useful information in orthopaedics and trauma practice. Measurements were performed on multiparametric automatic analyzer EOS Bravo Forte, Hospitex Diagnostics by wet chemistry with reagent kits in the laboratory Hospitex Diagnostics Platform for Interdisciplinary Research USAMVB of Timisoara. For determination of calcium, phosphorus, sodium and potassium the following dosing methods were used: Serum calcium was determined by colorimetric method with o-cresolphthalein without precipitation. Method for determining inorganic phosphorus was UV method based on phospho-molybdate formation using a Diagnostix Hospitex Monoreagent kit. Potassium Method: turbimetric method. Sodium Method colorimetric method. From the laboratory results obtained by us only the variation of plasma calcium and phosphorus values obtained on one hand and those of potassium and sodium on the other hand were interesting, otherwise other values are not significant. By assessment of calcium and phosphorus levels we notice a marked hypocalcemia at 14 days after surgery, which tends to reach normal levels at 21 days compared to phosphate which still shows a trend of decreasing its values and 21 days after surgery. We cannot draw a definitive conclusion on these experimental observations but they warrant further research on a larger lot along with their evolution in the clinic.

Keywords: wet chemistry, chemical elements, calcium, sodium, potassium, phosphorus, blood components, lesion syndrome.

The skeleton is an organ that is subject to constant remodeling and repair. Remodeling of the callus leads to restoration of the anatomic and biomechanical properties of the original skeletal element [1].

A number of factors have been shown to influence fracture repair, and analyses of the molecular basis for these abnormalities has led to a greater understanding of this process [2]. Callus formation is a complex biological process involving changes in the expression of many genes [3].

Surgery is represented in our study by creating a solution of continuity in the bones, followed by transplantation of autogenous or homogeneous bone grafts, producing a traumatic syndrome meaning by this "all local and systemic alterations caused by the actions of pathogen". [10], which in our case is represented by both the surgery and the bone grafting material.

Depending on the intensity of aggression, the body is mobilized, manifesting itself through a complex defence and compensation nonspecific reactions resulting in so-called syndrome reaction.

Experiments conducted on human or animal models reveals biological pathways for optimal bone healing after injury [12]. Healing begins at the ends of the fragments and bone periosteum and initially the mechanical quality of bone offering low stability [13].

It is known that the existence of abnormal amount of chemicals in the blood can lead to bone pathology, such as: hypophosphatemia leads to osteomalacia which affects bone recovery after injury [2]. Additional animal studies have demonstrated the importance of phosphorus, in conjunction with calcium, for bone development [5]. In the human body, circulating calcium is useful for some function in neuromuscular function in skeletal metabolism and in hemostasis [5].

The research focused on the integration of the bone transplant in the recipient and on the reactivation of the entire body for this purpose following the evolution of the plasma concentration of chemical elements from an animal after bone transplant surgery and that of host tissue first weeks after surgery. Of course, this assessment of certain changes in homeostasis, does nothing more than provide a look upon some isolated syndrome features. However, analysis of plasma concentrations of chemicals and blood after transplantation gave us some interesting aspects related to clinical can give us some useful information in orthopaedics and trauma practice.

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Experimental part

Material and methods

Measurements were performed on multiparametric automatic analyzer EOS Bravo Forte, Hospitex Diagnostics by wet chemistry with reagent kits in the laboratory Hospitex Diagnostics Platform for Interdisciplinary Research USAMVB of Timisoara.

For internal quality control the following reference materials produced by Hospitex Diagnostics, Italy were used: HD calibrator calibrator serum, normal control serum: HD normal serum, serum values pathological control: QC serum.

The research was conducted on a group of 4 dogs. During the transplantation bone samples were collected at regular intervals of 7 days. This has been followed by the assessment of the clinical evolution, calcium, phosphorus, potassium, sodium, ESR's, the number of erythrocytes, haemoglobin index, the number of leukocytes and neutrophils. (figs. 1-9). For determination of calcium, phosphorus, sodium and potassium were used following dosing methods.

Serum calcium was determined by colorimetric method with o-cresolphthalein without precipitation [9]. Normal values for dogs veterinary university clinics Timisoara were 6-8 mg calcium per 100 mL serum. The method for determining inorganic phosphorus was photometric UV method based on the formation of phospho-molybdate using a Diagnostix Hospitex Monoreagent kit [8]. The normal values in dogs are 5.5 to 7.6 mg in 100 mL serum inorganic phosphorus. Potassium levels were determined by turbidimetric method [10]. Normal levels are 3.5 to 4.5 mg/L. The sodium levels were determined by colorimetric method with normal levels = 146-159 mEg/L [11].

Results and discussions

Dosage of total calcium

The calcium levels in experimental animals are represented in figure 1.

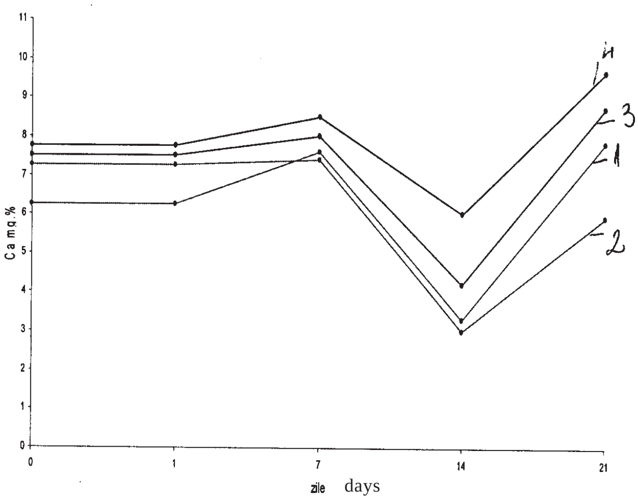

Fig.1. Calcemia in the first weeks after transplantation

The phosphorus levels in experimental animals are represented in figure 2.

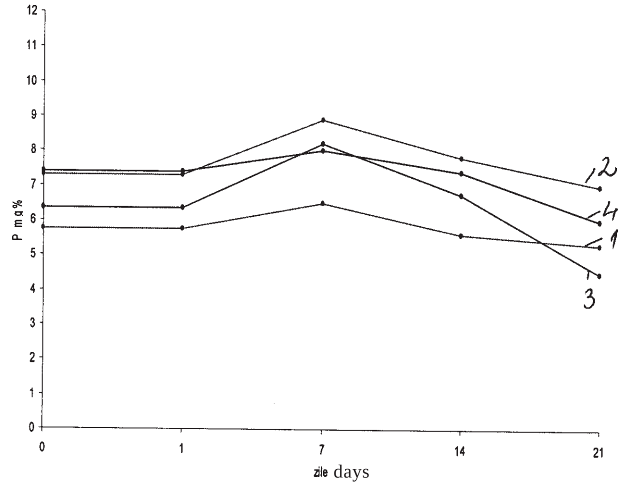

Fig.2. Evolution of phosphorus levels

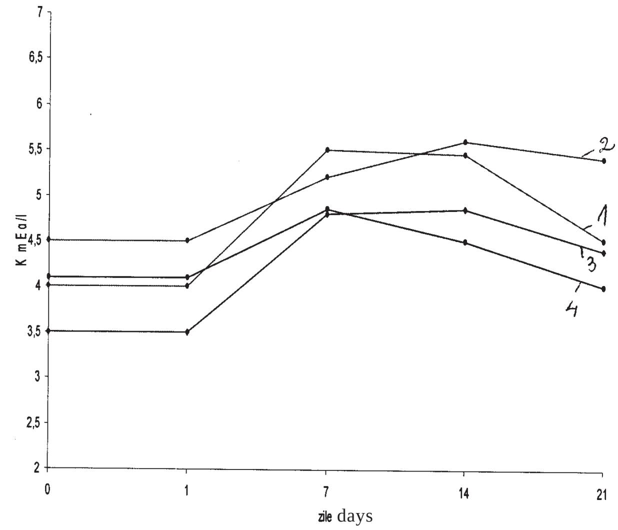

Fig 3. Evolution of potassium in the first weeks after transplantation.

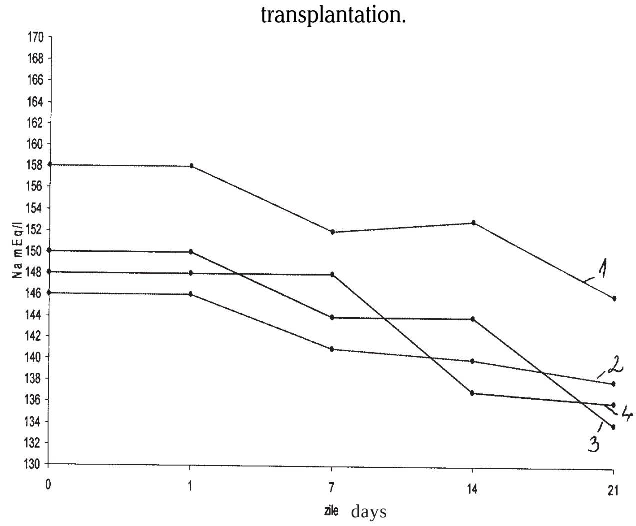

Fig.4. Sodium values

Dosage of sodium and potassium

The remaining tests were performed by conventional techniques and normal biological constants, followed in experimental animals, have been identified by the starting point of the graphics. Graphics were prepared separately for each biological constant traced to each of the four dogs. Experimental animals were represented on the charts after following legend:

1 - dog no.2
2 - dog No.3
3 - dog No.1
4 - dog no.4

Following the evolution of the ESR values of the four dogs postoperative interval of seven days, we see that at the end of the first week, in dogs No. 1 and 4, the values are higher, and in the same time dogs 2 and 3 show a slight increase from normal values. At 14 days the dog No.2 shows the highest value. At 21 days, all experience animals had declining values, while remaining high in dog 1, which also had elevated ESR in the first day (table 1).

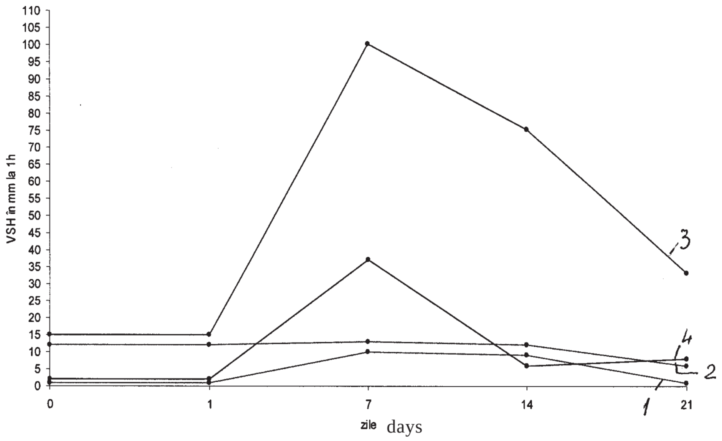

Fig 5. Evolution of ESR values after transplantation

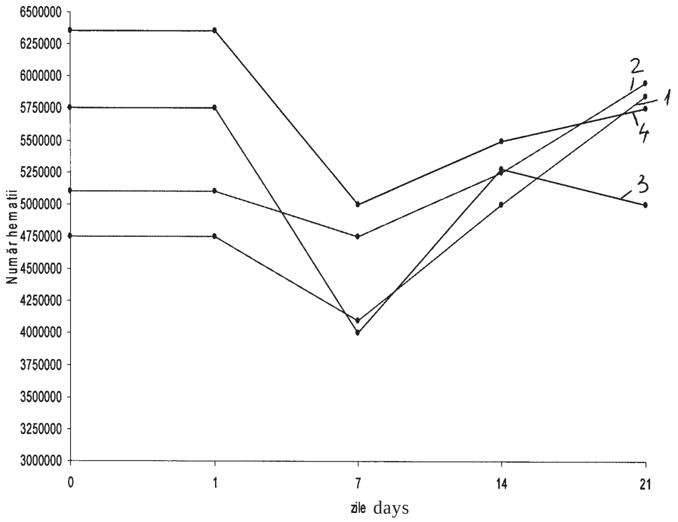

Fig 6. Evolution of red blood cells count (RBC)

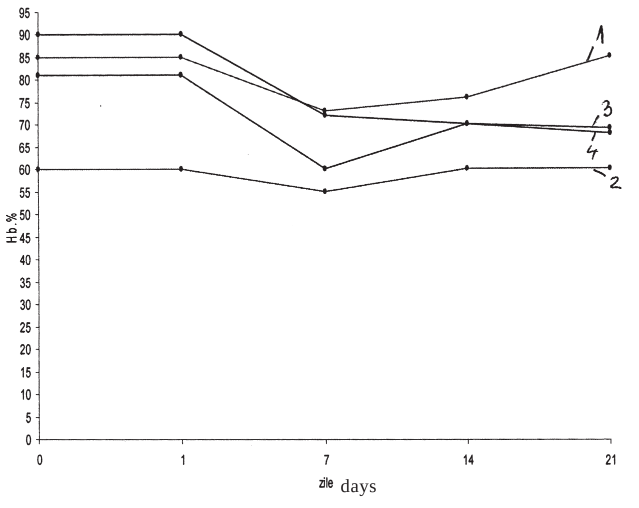

Fig.7. Evolution of the haemoglobin index

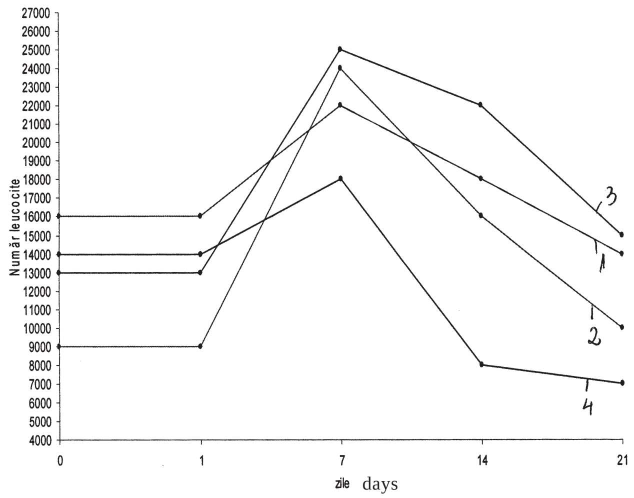

Fig 8. Evolution of the white blood cells count (WBC) in the first five weeks after transplanting

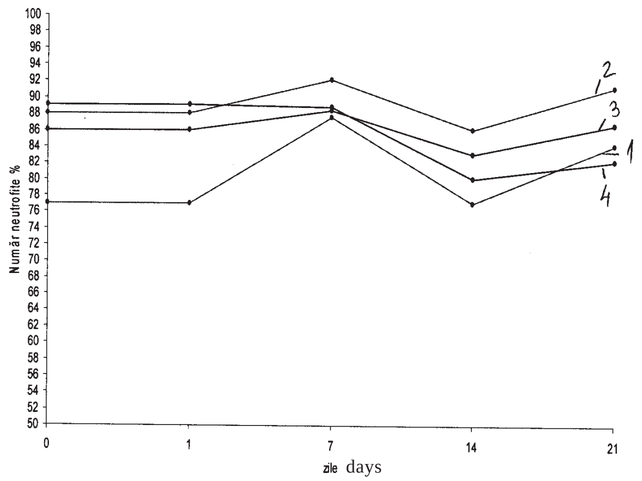

Fig 9. Evolution of the neutrophil count in the first weeks after transplantation

Calcium and phosphorus values give us the most interesting data in the evolution of their curves, and the values obtained are almost equal in all experimental animals, without any discordance. Serum calcium shows a slight increase at the end of 7 days after surgery, and then drops suddenly at 2 weeks. At 21 days we have noticed rise curves in all animals reaching normal levels in some, or even above normal, in the other experience animalss (table 2).

Unlike calcium, phosphorus followed an inverse trend. At the end of the first week, a slight increase is recorded in the entire experimental group, starting from day 7, then serum phosphorus levels follow a downward trend

0	1(day)	7(days)	14(days)	21(days)
1	1	10	9	1
3	3	37	6	8
12	12	13	12	6
15	15	100	75	33

Table 1
EVOLUTION OF ESR VALUES (mm to 1 h) AFTER TRANSPLANTATION.

0	1(day)	7(days)	14(days)	21(days)
6.2	6.2	7.6	3.3	7.8
7.3	7.3	7.4	3	5.9
7.5	7.5	8	5.9	8.7
7.7	7.7	8.5	6	9.7

Table 2
SERUM CALCIUM (mg%) IN THE FIRST WEEKS AFTER TRANSPLANTATION

0	1(day)	7(days)	14(days)	21(days)
5.8	5.8	6.5	5.7	5.3
6.4	6.4	8.2	7.4	6.0
7.3	7.3	8.9	7.8	7.0
7.4	7.4	8.0	7.3	5.9

Table 3
EVOLUTION OF PHOSPHORUS LEVELS (mg%) AFTER TRANSPLANTATION

0	1(day)	7(days)	14(days)	21(days)
3.5	3.5	4.75	4.85	4.40
4.00	4.00	5.50	5.40	4.50
4.10	4.10	4.80	4.50	4.00
4.50	4.50	5.25	5.20	5.40

Table 4
EVOLUTION OF POTASSIUM IN THE FIRST WEEKS AFTER TRANSPLANTATION

0	1(day)	7(days)	14(days)	21(days)
146	146	141	140	138
148	148	148	137	136
150	150	144	144	134
158	158	152	153	146

Table 5
EVOLUTION OF SODIUM IN THE FIRST WEEKS AFTER TRANSPLANTATION

0	1(day)	7(days)	14(days)	21(days)
4750000	4750000	4100000	5000000	5850000
5110000	5110000	4750000	5230000	5950000
5750000	5750000	4000000	5270000	5000000
6370000	6370000	5000000	5500000	5750000

Table 6
EVOLUTION OF RBC

0	1(day)	7(days)	14(days)	21(days)
60	60	55	60	60
81	81	60	70	69
85	85	73	76	85
90	90	72	70	67

Table 7
EVOLUTION OF THE HAEMOGLOBIN INDEX

0	1(day)	7(days)	14(days)	21(days)
9000	9000	24000	16000	10000
13000	13000	25000	22000	15000
14000	14000	18000	8000	7000
16000	16000	22000	18000	14000

Table 8
EVOLUTION OF WBC IN THE FIRST FIVE WEEKS AFTER TRANSPLANTING

reaching a maximum in the 21-day after intervention, reverse as that of calcium (table 3).

Potassium and sodium (table 4 and 5) also show interesting values such as: if potassium show high values in the first and second week, immediately from the first assessment sodium levels decrease massively, reaching 20 days to values well below normal ones.

Following the evolution of other biological constants and RBC, haemoglobin index, WBC and neutrophils number, RBC decrease with a maximum on seventh day, then values show a tendency to return to normal, haemoglobin index without any significance and increased WBC at the end of the first week with a tendency to return to normal in 21 days. Neutrophils show a slight increase to 7 days then their value is inconclusive (tables 6-9).

0	1(day)	7(days)	14(days)	21(days)
77	77	87.5	77	84
0	86	88.5	83	86.5
88	88	92	86	91
89	89	88.5	80	82

Table 9
EVOLUTION OF NEUTROPHILS NUMBER IN THE FIRST WEEKS AFTER TRANSPLANTATION

Conclusions

The exact mechanism by means of which calcium salts are deposited in areas of bone growth or bone repair is largely unknown [1]. Based on experimental research, producing closed fractures in dogs and watching the evolution of the ESR values, Baciu [4] concludes that at 2 weeks ESR all experimental animals increased significantly. Studying the biology of fixation with follow up of the evolution of biological constants in the case of fractures treated surgically and conservatively in experimental animals, the researchers [6, 7] found that the biological evolution of these constants was not conclusive.

The phosphate plays an important role in the skeleton that extends beyond mineralized matrix formation and growth plate maturation and is critical for endochondral bone repair [2].

Our results show that the evolution of the plasma concentration of calcium and phosphorus on the one hand and those of potassium and sodium on the other hand were interesting, the other having little significance.

Taking into consideration the levels of ESR, WBC and neutrophil count, we see that in our cases, they evolve towards a dysproteinemia with a probable contribution of fibrinogen and alpha-globulins.

Neutrophilic leukocytosis reach maximum values at 7 days after surgery. All this occurs as a nonspecific reaction.

The RBC decline with a lesser one in erythrocyte haemoglobin index around day 7 remain unexplained. The animals had no significant blood loss during surgery. Generally, all values assessed show a tendency to return to normal, starting on the 14th to the 21st day.

On calcium and phosphorus levels, we note a marked hypocalcemia at 14 days after surgery, which tends to reach normal levels at 21 days, compared to phosphoremia still showing a downward trend in its values and 21 days after the surgery. These data make us think of many variations that can clarify the occurrence of these phenomena.

Hypoparathyroidism, which may give a patho-physiological explanation for the relationship between calcium and phosphorus may be taken into consideration, and, on the other hand, the existence of a secondary reaction into the bone, assuming that the mineralization of the graft would increase the amount of calcium and phosphorus deposited into the graft.

Discussing values obtained for potassium and sodium and correlating them with the role of parathyroid hormone, which is involved in electrolyte metabolism, we could presume the involvement of the renal tubules as this hormone promotes sodium and bicarbonate loss in the urine and potassium retention.

If we accept that in this phase of hypocalcemia stimulation of parathyroid function occurs, which aims to restore serum calcium level, then we presume that changes in potassium and sodium as a secondary hiperparathyroidism, as shown in the graphs 2,4,5 in the first weeks after transplantation. (7-14 days)

All these data can be attributed to this dysfunction because clinically there was no evidence in experimental animals of cortico-adrenal insufficiency phenomena acute or chronic, which may be signs of a late lesion syndrome.

To note the role that transplantation would lead to general biological stimulus. The results from this experimental research can be related to clinical suggesting some useful information in orthopedic practice and trauma.

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