Calcium, phosphor and alkaline phosphatase value in bone graft calcium sulfate α-hemihydrate bioceramic application on animal bone defect (In Vivo Study)

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Abstract

Objective: This study aims to determine differences in the value of calcium, phosphorus, and alkaline phosphatase in the blood of bone graft material which is applied to bone defects.

Methods: 12 male rabbits were divided into 4 groups, then defects were made in the femur bone with a diameter of 5mm. Then each group was drawn with a vulnerable blood pre-operative time, 1 week, 3 weeks and 6 weeks after surgery.

Results: Average levels of calcium, phosphorus, and alkaline phosphatase in all treatment groups were higher than those in the control group.

Conclusion: There were differences in calcium levels in test animals using bone graft calcium sulfate hemihydrates bioceramic on days 7th, 21st and 42nd post surgery.

Keywords: Bone graft, Bone loss, Calcium sulfate

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Introduction

In the field of dentistry, alveolar bone reduction can be an obstacle in making artificial teeth.¹ ² Various methods of bone augmentation are done in an effort to increase bone volume. Autologous Bone Graft is the “gold standard” for bone grafting. But the amount is limited for each individual, and need to do additional operations.³ Another alternative is allograft, but it has a risk of transmission of the virus (HIV, hepatitis B, and C) from donor to recipient.⁴ Several alternatives have been developed for clinical use with various advantages including in the economic side. This is done by developing natural polymeric materials, synthesis, ceramics, and composites, or by combining with cells or various bone formation factors.

α-calcium sulfate hemihydrate (α-CSH, CaSO₄•0.5H₂O) is a bone graft replacement, and has been widely used in dental and orthopedic surgery, with good osteoconduct characteristics, good osteogenesis, and excellent biocompatibility.⁵ The latest combination of α-calcium sulfate hemihydrate (α-CSH, CaSO₄•0.5H₂O) with bioceramics has been carried out and tested in vitro. In vivo testing has not been carried out on these materials, so an in vivo test is needed by using various indicators in the process of bone formation to analyze this material. Calcium, phosphorus, and alkaline phosphatase in the blood is one indicator in the process of new bone formation.

Material and Methods

This study was conducted on 12 male rabbits, weighing 4-6 kg. Rabbits were divided into 4 groups. Group 1 (7th day interval), group 2 (21st day), group 3 (42th day), group 4 (control) each consisting of 3 animals. Surgery was performed on the lateral side of the femur bone which is made of a hole with a diameter of 5mm. Then the bone graft material was inserted into the bone defect, and suturing was done to the muscles and the skin. In the control group no bone graft was given on the defect that had been made. Blood samples were taken before taking action, and on the 7th, 21st, and 42th day after the operation according to the division of groups, for the control group taking blood was carried out at each time interval. Then blood samples were taken for examination and analysis of the value of calcium, phosphorus and alkaline phosphatase.

Results

All treatment groups will be presented in table 1-table 6 and figure 1-figure 4.
The average ALP in all treatment groups had a higher value than the control group. ALP levels increased on day 7, but began to decrease on days 21 to day 42. That ALP is a marker of bone formation, which can be induced by the addition of a particular material as a stimulator.\textsuperscript{1,6} The difference between this study and this research is the material used in the study, which uses chitosan as a scaffold. Significantly different comparisons were seen in the preoperative group compared with treatment on day 7, day 21, and day 42. Comparison between the preoperative group and treatment on day 7 had a significance value of 0.002 (p<0.005) which means that there is a significant difference between ALP levels at the preoperative day 7, where there is an increase. In addition there was a significant difference between the treatment group on day 7 and the treatment group on day 42, with a significance value of 0.000 (p<0.05). This shows that the addition of bone graft material α-calcium sulphate hemihydrates bioceramic has an influence in increasing the amount of ALP in the blood during bone formation.

These results indicate that there is an increase in ALP levels along with the increase in observation days. This is in line with research conducted by Saraswathy et al.\textsuperscript{7} which explained that there was

**Discussion**

| Table 1  | Alkaline phosphatase examination result |
|----------|---------------------------------------|
| Group(n=3)| Alkaline Phosphatase | Control |
| Pre-op | 46 | 42 |
| 1 | 90 | 87.7 |
| 2 | 37.7 | 36 |
| 3 | 36 | 31.67 |

| Table 2  | Calcium examination result |
|----------|-----------------------------|
| Group(n=3)| Calcium | Control |
| Pre-op | 3.57 | 3.54 |
| 1 | 3.98 | 3.94 |
| 2 | 3.87 | 3.79 |
| 3 | 3.91 | 3.86 |

| Table 3  | Phosphor examination result |
|----------|-----------------------------|
| Group(n=3)| Phosphor | Control |
| Pre-op | 1.52 | 1.57 |
| 1 | 1.71 | 1.51 |
| 2 | 2.95 | 2.85 |
| 3 | 4.01 | 3.74 |

Figure 1 A. Skin incision, B. Exposed bone, C. Defect was made, D. Bone defect, E. Bone graft application

Figure 2 Mean of ALP

Figure 3 Mean of calcium

Figure 4 Mean of phosphor
an increase in the amount of ALP in bone defects induced with bone graft material in the form of bio-inorganic composites. In addition, according to Saraswathy and colleagues, an increase in ALP levels after trauma to the bone may be related to the proliferation of osteogenic cells. That bone formation in crystalline composites as scaffold can induce bone formation by expression of ALP markers.

## Table 4 Analysis of ALP in blood on pra surgical day, day 7th, 21st and 42nd

| ALP     | (i)day | (j)day | Mean diff (i-j) | Std error | sig  | Lower bound | Upper bound |
|---------|--------|--------|-----------------|-----------|------|-------------|-------------|
| Tukey HSD Pre OP | Day 7th | -34.53333* | 6.63744 | 0.002 | -57.5231 | -11.5535 |
| Day 21st | 10.13333 | 6.63744 | 0.783 | -12.8465 | 33.1131 |
| Day 42nd | 10.80000 | 6.63744 | 0.729 | -12.1798 | 33.7798 |
| Control  | 1.80000 | 6.63744 | 1.000 | -21.1798 | 24.7798 |
| Day 7th Pre OP | 34.53333 | 6.63744 | 0.002 | 11.5535 | 57.5253 |
| Day 21st | 44.66667* | 6.63744 | 0.000 | 21.6869 | 67.6465 |
| Day 42nd | 45.33333* | 6.63744 | 0.000 | 22.3535 | 68.3131 |
| Control  | -5.33333 | 6.63744 | 0.990 | -28.3131 | 17.6465 |
| Day 21st Pre OP | -10.13333 | 6.63744 | 0.783 | -33.1131 | 12.8465 |
| Day 7th | -44.66667* | 6.63744 | 0.000 | -67.6465 | -21.6865 |
| Day 42nd | 0.66667 | 6.63744 | 1.000 | -22.3131 | 23.6465 |
| Kontrol  | 3 | 6.63744 | 0.999 | -19.6465 | 26.3131 |
| Day 42nd Pre OP | -10.80000 | 6.63744 | 0.729 | -33.7798 | 22.1313 |
| Day 7th | -45.33333* | 6.63744 | 0.000 | -68.3131 | -22.3535 |
| Day 21st | -0.66667 | 6.63744 | 0.990 | -23.6465 | 22.3131 |
| Kontrol  | 5.33333 | 6.63744 | 0.999 | -17.6465 | 28.3131 |

## Table 5 Analysis of calcium in blood on pra surgical day, day 7th, 21st and 42nd

| Calcium | (i)day | (j)day | Mean diff | Std error | sig  | Lower bound | Upper bound |
|---------|--------|--------|-----------|-----------|------|-------------|-------------|
| Tukey HSD Pre OP | Day 7th | 0.51333* | 0.09765 | 0.002 | -0.8514 | -1.1752 |
| Day 21st | -0.24000 | 0.09765 | 0.279 | -0.5781 | 0.0981 |
| Day 42nd | -0.44000* | 0.09765 | 0.007 | -0.7781 | -0.1019 |
| Kontrol  | -1.0000 | 0.09765 | 0.964 | -0.4381 | 0.2381 |
| Day 7th Pre OP | 0.51333* | 0.09765 | 0.002 | 0.1752 | 0.8514 |
| Day 21st | 0.27333 | 0.09765 | 0.163 | -0.0648 | 0.6114 |
| Day 42nd | 0.07333 | 0.09765 | 0.994 | -0.2648 | 0.4114 |
| Kontrol  | 0.04000 | 0.09765 | 1.000 | -0.2981 | 0.3781 |
| Day 21st Pre OP | 0.24000 | 0.09765 | 0.279 | -0.0981 | 0.5781 |
| Day 7th | -0.27333 | 0.09765 | 0.163 | -0.6114 | 0.0648 |
| Day 42nd | -0.20000 | 0.09765 | 0.483 | -0.5381 | 0.1381 |
| Kontrol  | -0.8667 | 0.09765 | 0.983 | -0.4248 | 0.2514 |
| Day 42nd Pre OP | 0.44000* | 0.09765 | 0.007 | 0.1019 | 0.7781 |
| Day 7th | -0.07333 | 0.09765 | 0.994 | -0.4114 | 0.2648 |
| Day 21st | 0.20000 | 0.09765 | 0.483 | -0.1381 | 0.5381 |
| Kontrol  | 0.04667 | 0.09765 | 1.000 | -0.2914 | 0.3848 |

Analysis of the ALP amount was carried out using the ALP assay which was read through absorbance. Minli X et al. colleagues also explained that biocomposites can stimulate bone formation through a process of mineralization mediated by ALP which appears as a marker by the presence of composite stimulation combined with calcium phosphate. In addition, Chen and colleagues explained that the expression of ALP, COL1, OPN, OCN and RUNX2 was very high at weeks 3 through 6 in stem cell osteogenesis.
induced with α-hemihydrate calcium sulphate with a combination of sodium hyaluronate. In that study, complete bone healing in the implant area around 9 weeks. That α-calcium sulphate hemihydrates containing cerium can induce osteogenesis by the expression of alkaline phosphatase, Runx2, BSP and OCN. Liu and colleagues also explained that α-calcium sulphate hemihydrates containing Strontium has the ability to repair bone through the stimulation of the expression of Runx2, alkaline phosphatase, OCN and BSP both in vitro and in vivo.

In the analysis of average calcium levels, it was found that all treatment groups had higher values than the control group. Calcium levels increased on day 7, but decreased on day 21 and increased again on day 42. This is in line with research by Saraswathy and colleagues who stated that there was a decrease in calcium levels until day 21, but experienced an increase again in until day 42, but not higher than calcium levels during the preoperative period. According to him, this condition is caused by increased urinary excretion after trauma to the bone.

Significant differences were only seen in two groups, namely the preoperative group with treatment on day 7 and the preoperative group with treatment group on day 42. The preoperative group with treatment group on day 7 had significant differences with a significance value of 0.002 (p<0.05). This shows that the increase in calcium levels from the preoperative period to the 7th day is significant. The preoperative group with the treatment group on day 42 had a significant difference with a significance value of 0.007 (p<0.05).

That calcium levels in the preoperative period when compared to calcium levels on day 42 showed a significant significant difference, indicating that the addition of bone graft α-calcium sulphate hemihydrates bioceramic materials had an influence in increasing the amount of calcium in the blood during bone formation. This is different from research conducted by Saraswathy, in which the preoperative calcium level was slightly higher than on day 42. Research conducted by Keya et al. also showed mineralization at new bone encounters with the addition of α-calcium sulphate hemihydrates, which showed increased levels of calcium ions compared to normal bone tissue. Liu and colleagues also obtained results in the form of elevated levels of calcium and collagen fibers in the bone which had healing until week 8.

At the average phosphorus level, all treatment groups had higher values than the control group. Calcium levels increased on day 7, and continued to increase from day 21 to day 42. A significant difference was seen in the preoperative group compared with treatment on day 21, and day 42. In addition, there were differences significant between groups on the 7th day with the 21st day, 7th day with 42nd day, and 21st day with 42nd day. Comparison between the preoperative and treatment groups on 21st day has a significance value of 0.000 (p<0.005) which means that there is a significant difference between calcium levels in the preoperative group with day 21.

| Phosphor (i)day | (j)day | Mean diff | Std error | sig | Lower bound | Upper bound |
|----------------|--------|-----------|-----------|-----|-------------|-------------|
| Tukey HSD Pre OP | Day 7th | -0.22667  | 0.22653  | 0.968 | -1.0109  | 0.5576      |
|                | Day 21st | -1.47333* | 0.22653  | 0.000 | -2.2576  | -0.6891     |
|                | Day 42nd | -2.53333* | 0.22653  | 0.000 | -3.3176  | -1.7491     |
|                | Control  | -0.08000  | 0.22653  | 1.000 | -0.8643  | 0.7043      |
| Day 7th        | Pre OP   | 0.22667   | 0.22653  | 0.968 | 0.5576   | 1.0109      |
|                | Day 21st | -1.24667* | 0.22653  | 0.001 | -2.0309  | -0.4624     |
|                | Day 42nd | -2.30667* | 0.22653  | 0.000 | -3.0909  | -1.5224     |
|                | Control  | 0.20000   | 0.22653  | 0.984 | -0.5843  | 0.9843      |
| Day 21st       | Pre OP   | 1.47333*  | 0.22653  | 0.000 | 0.6891   | 2.2576      |
|                | Day 7th  | 1.24667*  | 0.22653  | 0.001 | 0.4624   | 2.0309      |
|                | Day 42nd | -1.06000* | 0.22653  | 0.005 | -1.8443  | -0.2757     |
|                | Control  | 2.45333*  | 0.22653  | 1.000 | -0.6843  | 0.3757      |
| Day 42nd       | Pre OP   | 2.53333*  | 0.22653  | 0.000 | 1.7224   | 3.3176      |
|                | Day 7th  | 2.30667*  | 0.22653  | 0.000 | 1.5224   | 3.0909      |
|                | Day 21st | 1.06000*  | 0.22653  | 0.005 | 0.2757   | 1.8443      |
|                | Control  | 0.27333*  | 0.22653  | 0.918 | -0.5109  | 1.0576      |
In addition there was a significant difference between the treatment group in the preoperative group and the 42nd day group, with a significance value of 0.000 (p<0.05). There was also a significant difference between the treatment group on day 7 with the treatment group on day 21, with a significance value of 0.001 (p <0.05), and a significant difference between the treatment group on day 7 with day 42 with a significance value of 0.000 (p < 0.05). In addition, there was a significant difference between the 21st day and 42nd day groups with a significance of 0.005 (p<0.05). This indicates that the addition of bone graft material α-calcium sulphate hemihydrates bioceramic has an influence in increasing the amount of phosphorus in the blood during bone formation.

This is in line with research conducted by Bonjour, which states that calcium and phosphorus are the two main ions that replace hydroxyapatite, bone minerals that strengthen the mechanical resistance of the organic matrix that signifies the process of bone formation. In line with Saraswathy’s research which explains that there are increased levels phosphorus from the preoperative day to the 21st day but decreased on day 42. This study also found conditions where there was an increase in phosphorus levels until the 21st day. However, in contrast to his research, in this study, phosphorus levels continued to increase until the 42nd day According to him, the increase or decrease in phosphorus levels in the healing period is not significant, and the mechanism is unknown.

Conclusion
There was a significant difference in the value of calcium, phosphorus and alkaline phosphatase in test animals using α-calcium sulphate hemihydrates bioceramic bone graft on days 7, 21 and 42 postoperatively compared with no additional bone graft.

Acknowledgment
None

Conflict of Interest
The authors report no conflict of interest.

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