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The ability of $Ca(OH)_2$ to facilitate bone formation by measuring the alkaline phosphatase level (Experimental study)

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ABSTRACT

Aim: To investigate the early events of bone regeneration of $Ca(OH)_2$ by studying biochemistry level of alkaline phosphatase (ALP) as guide for bone regeneration. **Materials and Methods:** The mandible of eight dogs was prepared by hole of 0.5×0.5 cm in depth and diameter in the apical to the molar teeth. This hole packed with $Ca(OH)_2$ powder mixed with distilled water to become as a paste. Samples of blood were collected pre–operatively considered as control groups [measuring level of serum ALP in experimental animals before implanted $Ca(OH)_2$], then at the 7, 10 and 15 day postoperatively considered as treated groups [measuring alteration of level of serum ALP in experimental animals after implanted $Ca(OH)_2$] for biochemical analysis of the level of serum ALP. **Results:** There was a significant difference at the day 10 following the operation between the control groups and the treated groups, while there was no significant difference at the day 7,and day 15 postoperatively between the control and the treated groups. Also the results showed that there was a high significant difference between the different postoperative days. **Conclusion:** The $(CaOH)_2$ powder mixed with distilled water have the ability to accelerate bone regeneration when it is placed in bone defect comparing to that defect filled with nothing.

Key Words: Calcium hydroxide, alkaline phosphatase, dogs.

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INTRODUCTION

Alkaline phosphatase (ALP) found in high concentration in liver, bone, intestine, placenta and kidney. The main source of serum enzyme is hepatobiliary tree and osteoblast. The cause of increased serum ALP activity are either physiological like infancy, puberty and pregnancy because during this period there is increase in osteoblastic activity due to the growth of bone, or pathologically elevated in case of bone and hepatobiliary diseases. However, markedly reduced levels are found in the inherited condition like in hypophosphatasia which is caused by defective bone calcification.[1]

The ALP enzyme is essential for mineralization of bone, the calcification of the matrix is still controversial. However, the precipitation of calcium salts is the product of the local concentration of calcium and phosphate ions.

The calcium and phosphate products when the product reaches a certain level, tiny crystals of hydroxyapatite form spontaneously. This form the nuclei for further crystallization by the help of matrix protein such as osteonectin and osteocalcin that bind and concentrate calcium.[2]

The ALP enzyme essential for mineralization either by directly stimulates calcium salts deposit or acts indirectly by blocking the action of calcification inhibitor.

The plasma membrane of osteoblasts and chondroblasts bear the enzyme, when this enzyme pinches off and enter the matrix calcium salt precipitation occurs. It plays a role in the remodeling process and healing of fracture and bone transplantation by precipitation of calcium salt and local super saturation of phosphates leading to calcification of new bone.[3]

Serum biochemical markers of bone formation, such as ALP activity, may be clinically useful in evaluating the progress of healing.[4]

Calcium hydroxide stimulates tissue repair by inducing the formation of miner-

alized tissue.[5, 6] Calcium hydroxide is also effective against microorganisms present in the root canal.[7–11] The influence of elevated pH of calcium hydroxide on bacterial and tissue enzymes reinforces the importance of the biological and chemical dynamics since its properties derived from the disassociation of calcium and hydroxyl ions,[12] explaining its mechanism of action, report that the effect of its pH alters the conveyance of nutrients and organic components through the cytoplasmic membrane, inhibiting enzymatic activities which are essential to bacterial life, such as metabolism, growth, and cellular division, and liberating a toxic action which is harmful to bacteria. It also activates ALP, a hydrolytic enzyme intimately related to the process of tissue mineralization. For these reasons, the authors believed that calcium hydroxide presents two essential enzymatic properties: Inhibition of bacterial enzymes for its antibacterial effect and activation of tissue enzymes, such as ALP, leading to its mineralizing effect.

So, this study was conducted to investigate the early events of bone regeneration of $Ca(OH)_2$ by studying biochemistry level of ALP as guide for bone regeneration.

MATERIALS AND METHODS

Eight dogs from both sexes, weight 16–24 Kg aged 6 month to 2 year apparently healthy were used in this study. All animals were examined and prepared in one week before the surgical operation. Animal kept in cages in D epartment of Veterinary Surgery with food and water supply daily.

All animals were fasted before day of operation to prepare them for general anesthesia using Ketamine HCl dose (15 mg/Kg body weight) with Xylzine HCl dose (5 mg/Kg body weight) giving intramuscular.

Sample of blood of about 1–1.5 ml was aspirated from the femur vein and considered as preoperative measures. Then other samples of blood were collected at the 7, 10 and 15 postoperative days for biochemical analysis of the level of serum ALP. The procedure for measuring serum ALP followed the direction of use by Biomerieux Vitek, Inc/ France, set up the following tubes:

	Serum Sample	Serum Blank	Standard	Reagent Blank
Reagent 1	2 ml	2 ml	2 ml	2 ml
Incubate for 5 minutes at 37° C				
Serum	50 µl	-----	-----	-----
Reagent 2	-----	-----	50 µl	-----
Incubate for exactly 15 minutes at 37° C				
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mix well or preferably vortex				
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	-----	50 ul	-----	-----
Distilled water	-----	-----	-----	50 ul

Mix let stand for 10 minutes in the dark

$$\text{Calculation: } \frac{\text{OD serum sample–OD serum blank}}{\text{OD standard}} \times n$$

Wave length: 510nm (Hg 492) by spectrophotometer.
Zero adjustment: Reagent blank.
OD =optic density n = Concentration of standarad.

The surgical site was sterilized by iodine solution, then a 3 sided flap was incised in the mandibular posterior region at the molars area. The flap was reflected to expose the alveolar bone, then a hole of 0.5×0.5 cm in depth and diameter was prepared in the mandible apical to the molar teeth using surgical hand piece and round bur with distilled water irrigation. Then, the surgical site was irrigated and cleaned. This hole then packed with $Ca(OH)_2$ powder mixed with distilled water to become as a paste, and the flap was repositioned and sutured. The same surgical procedure was performed for experimental 6 animals and considered as treated groups. The same surgical operation was performed to other 2 animals but the hole is left empty without anything and considered as a control group. Further investigation using cephalometric radiograph for the dog jaw, to compare the degree of radioopacity in the subsequent follow–up days (Figures 1 and 2).

Regarding statistical analysis, data were submitted to the statistical analysis using independent sample test (Student's t–test), one way analysis of variance (ANOVA) and Duncan's Multiple Range Test.

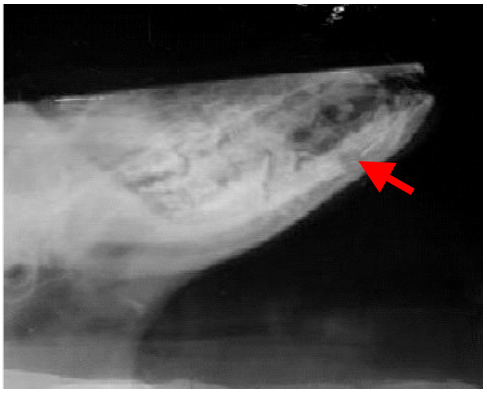

Figure (1): Well circumscribed radiolucency of hole implanted with $Ca(OH)_2$ at day 7 post-operatively as shown by arrow

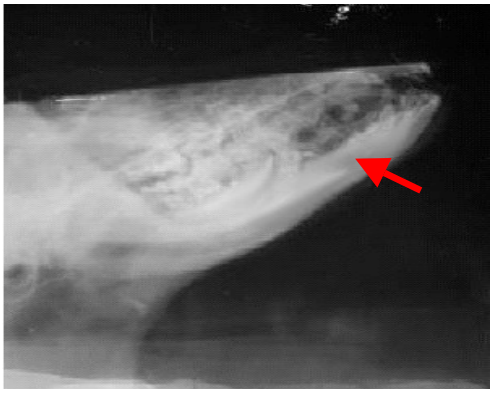

Figure (2): No clear demarcation of radiolucency of hole implanted with $Ca(OH)_2$ at day 10 post-operatively as shown by arrow

RESULTS

Table (1) and Figure (3) showed that there was a significant difference at the day 10 following the operation between the control and the treated groups of animals, while there was no significant difference at the day 7 and day 15 postoperatively between the control and the treated groups.

When ANOVA was done on the treated group, it showed that there was a significant difference between the different postoperative days ($p \leq 0.05$). Following the Duncan's Multiple Range Test was done on the treated group, it showed that there was a significant difference between the 10 and 15 postoperative days of the treated group (Table 2 and Figure 4).

Table (1): Relation between the control and treated groups at different postoperative days

	Control				Treated				*p*–value
	No.	**Mean (µ/L)**	**± SD**	**SE**	**No.**	**Mean (µ/L)**	**± SD**	**SE**	
Day 7	2	19	1.414	1.000	6	18	4.335	1.770	0.770
Day 10	2	20	1.323	1.000	6	30	4.388	1.624	0.045*
Day 15	2	15	0.000	0.000	6	12.583	3.638	1.485	0.407

No.: Number; SD: Standard deviation; SE: Standard error.
*Significant at the level ($p \leq 0.05$).

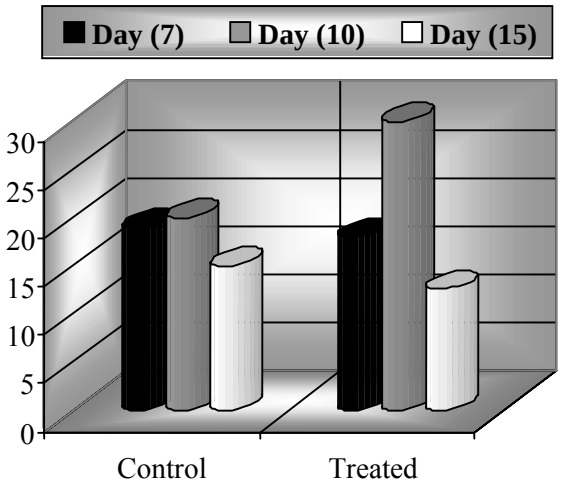

Figure (3): Relation between the control and treated groups at different postoperative days

Table (2): Analysis of variance and Duncan's Multiple Range Test for the relation of different postoperative day of the treated group

	df	Sum of Squares	Mean Square	F–value	*p*–value
Between Groups	3	918.865	306.288	24.999	0.000
Within Groups	20	245.042	12.252		
Total	23	1163.906			

df: Degree of freedom.

	No.	Mean ± SE	Duncan's Grouping*
Day 7	6	18.00 ± 1.770	B
Day 10	6	30.00 ± 1.624	A
Day 15	6	12.583 ± 1.485	C

No.: Number; SE: Standard error.
*Means with different letters is significant at 0.05 level.

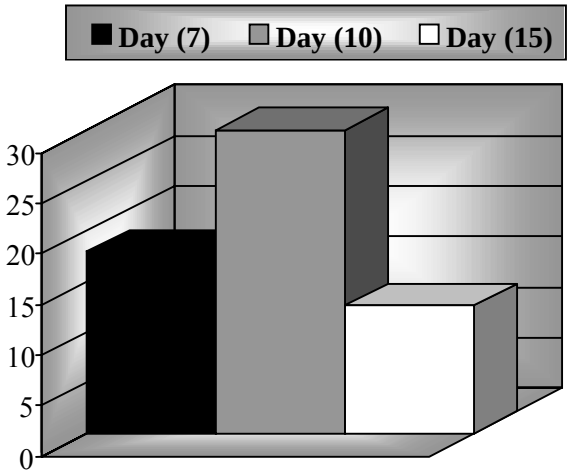

Figure (4): The relation of different postoperative day of the treated group

DISCUSSION

Bone defects and regeneration are important clinical considerations in oral and maxillofacial and reconstructive surgery occur in a wide variety of clinical situations, and successful closure of these defects remains as a major concern in the early phase of bone healing as which organization of the clot is completed can be followed clinically, whereas bone remodeling can be determined biochemistry via ALP or radiographically.[13, 14]

The ALP level express an early sign of osteoblast secretion of matrix which subsequently determined to become osteiod. This activity never been isolated from connective tissue away from bone thus indicated that its source is limited to existing bone. For this physiological properties, designed ALP as guide for bone regeneration.

The ALP catalyzes the hydrolysis of phosphate esters and it seems to be a prerequisite for normal skeletal mineralization. Also, it is the most widely recognized marker of osteoblast phenotypes by a tissue regenerative technique called Guided Bone Regeneration (GBR). It is possible nowadays to regenerate small bony defects.[15]

In this study, the results showed that the level of ALP in the blood was increased significantly at 10 postoperative days comparing with control animals ($p \leq 0.05$). This finding was in agreement with Gungormus and Kaya.[16] Their study were designed firstly on experimental animals (rabbits) showed that increased ALP corresponding to radioopacity (investigate radiographically), and secondly on patients volunteers do apicectomy. The latter provide a more rapid regeneration of bone defect of 3 months instead of 5 months compare to control group, since the authors used large periapical surgical defect for repairing like cyst and post–extraction defect.

Another agreement with experimental study on lab rats $Ca(OH)_2$ accelerate dimensional reduction of experimental cavities in which post–extraction defect used as experimental cavity. They led to good quality ossifications with new bone regeneration reaching from the vital margins of the defect concentrically towards the center.[17]

The ability of $Ca(OH)_2$ to enhance bone regeneration attributed to the fact that rise of alkalization in the tissue during present time to pH value around 10.5. This bring about, on the other hand, the differentiation and the growth of osteoblast thereby bossing bone regeneration.[18]

No infection or other complications occurred suggested that $Ca(OH)_2$ has a good tolerability. This attributed to anti–inflammatory and antibacterial effect of $Ca(OH)_2$.[19]

The ability of $Ca(OH)_2$ in stimulating this occurrence was reported in several studies in dentistry. They observed calcification of the pulp root canals in experimental dogs', and human teeth at the time range from 10 to 15 days after treatment.[20, 21]

In current study, the follow–up was carried out for short interval of time between subsequent days (7–10–15 days). This is due to evaluate the early sign of inducing bone by Ca(OH)2 as that reported by Shteyer *et al*.[22]

CONCLUSION

From the results of this study, it could be concluded that $Ca(OH)_2$ powder mixed with distilled water have the ability to accelerate bone regeneration when it is placed in bone defect comparing to that defect filled with nothing.

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