

## COMPARATIVE EVALUATION OF SURGICAL MANAGEMENT OF LONG BONE FRACTURE WITH INTERLOCKING NAILING ALONE AND ALONG WITH $\beta$ -TRICALCIUM PHOSPHATE IN DOGS

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### ABSTRACT

The goal of the current study was to assess the effectiveness of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) in healing of bone in dogs with long bone fractures repaired using interlocking nail technique. Twelve dogs regardless of age, breed, or sex were chosen and divided into two groups at random. Six canines were enrolled in group I, and interlocking nailing was done on them while six dogs were included in group II where  $\beta$ -TCP was placed over fractured site after the fracture was stabilized with interlocking nailing. Postoperatively, clinical and radiographic examinations were done on days 15<sup>th</sup>, 30<sup>th</sup> and 60<sup>th</sup>. At different times during the study, group II performed better than group I in terms of mean weight bearing and mean bone union scores. The results of the current study indicate that  $\beta$ -TCP efficiently speeds up bone healing in dogs who have long bone fracture.

**Keywords:**  $\beta$ -Tricalcium Phosphate, Fracture, Interlocking nailing

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A fracture will always result in pain and suffering, in addition to the injured limb losing its function (Vardhan *et al.*, 2017). Weight-bearing long bone fracture typically results from severe trauma, such as a vehicle accident (Huang *et al.*, 2012). The goal of fracture treatment is to facilitate the patient's earliest ambulation and the fastest healing feasible (Shahar, 2000). Long bone fracture in dogs is repaired using both conservative and operative techniques (Brinker *et al.*, 1994). Compared to repair methods used outside of the bone, the interlocking nail's placement within the medullary canal offers a biomechanical benefit because it is aligned with the construct's neutral axis (Dueland *et al.*, 1996).

Alloplasts, often known as "bone substitute materials," are inert synthetic graft materials that comprise tricalcium phosphate (TCP) and synthetic hydroxyapatite ceramics. These substances only have osteoconduction as their mode of action. They serve as a scaffold for improved bone tissue growth and healing (Liu and Kerns, 2014). Ceramics made of calcium phosphate, which are common in native human bone, have started to be recognised as appropriate biomaterials (Kanazawa *et al.*, 1975). It has been noted that calcium phosphates have osteoconductive and osteoinductive properties, and they support mesenchymal stem cells' osteogenic development (Shih *et al.*, 2014). This ceramic's ability to increase adenosine signalling in phosphate metabolism and provide

osteoinductive growth factors has been proposed as a way to enhance osteogenesis (Hoppe *et al.*, 2011; Shih *et al.*, 2014). The function of osteoclasts is inhibited by agonists of the Adenosine A2A receptor, which is strongly related to bone metabolism. Adenosine A2A receptor agonists can also activate osteoblasts and attract mesenchymal stem cells to the bone marrow (Lopez *et al.*, 2019). Additionally, it is believed that  $\beta$ -TCP has a favourable impact on the expression of the gene for bone morphogenetic protein (BMP-2) (Tang *et al.*, 2017). So, the present study was undertaken to evaluate the effects of Beta-tricalcium phosphate on bone healing along with interlocking nail in canines.

### MATERIALS AND METHODS

The 12 dogs used in this study were brought to the department for treatment of long bone fractures. They were separated into two groups at random, regardless of their age, breed, sex, or body weight, as shown in Table 1.

**$\beta$ - tricalcium phosphate ( $\beta$ -TCP):**  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) granules having a particle size range of 355 to 500 $\mu$  were employed as an osteoconductive material to fill the bone defect at the fracture site. Prior to surgery, all the animals were administered intramuscular injections of prophylactic antibiotic Ceftriaxone @ 25 mg/kg body weight, @ 0.04 mg/kg of Atropine Sulphate, and @ 0.2 mg/kg of Meloxicam as pre-emptive analgesia. After waiting for five minutes, an intramuscular injection

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of a combination of xylazine hydrochloride (1 mg/kg body weight) and ketamine hydrochloride (5 mg/kg body weight) was given. After the swallowing reflex was lost, the patient underwent endotracheal intubation, and isoflurane anaesthesia was maintained for the remainder of the surgery at a flow rate of 1.0-3.0%.

**Surgical technique:** The femur, tibia, and humerus were approached for interlocking nailing in the current study. Fracture fragments were lifted with a bone hook and held with bone holding forceps once the fracture site had been made visible. A tampon was used to remove the haematoma at the fracture site and detach the muscles' adhesion to the fracture fragments. For the femur, humerus, and tibia in both groups, interlocking nails of suitable diameter were placed normogradely through the medullary cavity. An L-shaped structure with a threaded knob is called an external aiming device (Jig), and it can be used to align screws with holes in nails. Additionally, intraoperative C-Arm use was used to ensure anatomical reduction and the correct pin position.

In group II, 1 cc of sterile beta-tri calcium phosphate bone graft was deposited in a sterile petridish mixed with NSS and administered using a spatula at the fracture gap after anatomical reduction and internal fixation in a similar manner. As per conventional procedure, the skin was closed with silk no. 1 and the muscles with Vicryl no. 1.

Post-operatively meloxicam was advised @ 0.3 mg/kg body weight I/M and Ceftriaxone @ 25 mg/kg body weight I/M for 5 days. After the operation, a modified Robert Jones bandage was put on to support the operated limb, and it was suggested that you walk on a leash for two weeks. After 15 days following surgery, the skin sutures were taken out.

**Post-operative evaluation:** Clinical and radiographic evaluations were performed at day 0, 15<sup>th</sup>, 30<sup>th</sup> and 60<sup>th</sup> following surgery to assess postoperative bone healing. Weight bearing while standing and walking was evaluated according to the method recommended by Sahu *et al.* (2017) and has been presented in Table 2. In accordance with Lane and Sandhu (1987), radiographic assessments were performed to assess bone formation scores and bone union scores (Table 3), and the stage of bone union (Table 4) was assessed in accordance with Hammer *et al.* (1985). A classification system adopted by Fox (1995) was used to evaluate clinical outcomes based on functional limb usage (Table 5).

**Statistical analysis:** The statistical analysis of data was done by one-way-ANOVA with linear repeated measure by SPSS software. All the data values were expressed as Mean  $\pm$  Standard error of mean (Mean $\pm$ S.E.). P-value less

than 0.05 considered as statistically significant.

## RESULTS AND DISCUSSION

In dogs with long bone fractures fixed using interlocking nails, this study was done to determine the

**Table 1. Design of the study**

Group	Treatment plan
Group I (n=6)	Internal fixation using interlocking nailing
Group II (n=6)	Internal fixation using interlocking nailing along with $\beta$ -TCP at fracture site

**Table 2. Weight bearing scoring system while standing and walking (Sahu *et al.*, 2017)**

Score	Description
<b>Weight bearing while standing</b>	
0	Carrying the limb off the ground
1	Touching the toe on the ground
2	Touching the paw on the ground
3	Full weight bearing
<b>Weight bearing while walking</b>	
0	Carrying the limb off the ground
1	Occasional touching of toe/paw on each step
2	Frequent touching of toe/paw on each step
3	Touching the toe on every step
4	Touching the paw on every step

**Table 3. Radiographic scoring system (Lane and Sandhu, 1987)**

Score	Description
<b>Bone Formation Scores</b>	
0	No evidence of bone formation
1	Bone formation in 25% of the gap
2	Bone formation in 50% of the gap
3	Bone formation in 75% of the gap
4	Bone formation in 100% of the gap
<b>Bone Union Scores</b>	
0	With complete fracture trace
2	With incomplete fracture trace
4	Absence of fracture trace

**Table 4. Stage of bone union radiographic scoring system (Hammer *et al.*, 1985)**

Grade	Callus Formation	Fracture Line	Stage of Union
1	Homogenous bone structure	Obliterated	Achieved
2	Massive- Bone trabeculae crossing the fracture line	Achieved	Barely discernible
3	Apparent-Bridging of fracture line	Discernible	Uncertain
4	Trace- No bridging of fracture line	Distinct	Not Achieved
5	No callus formation	Distinct	Not Achieved

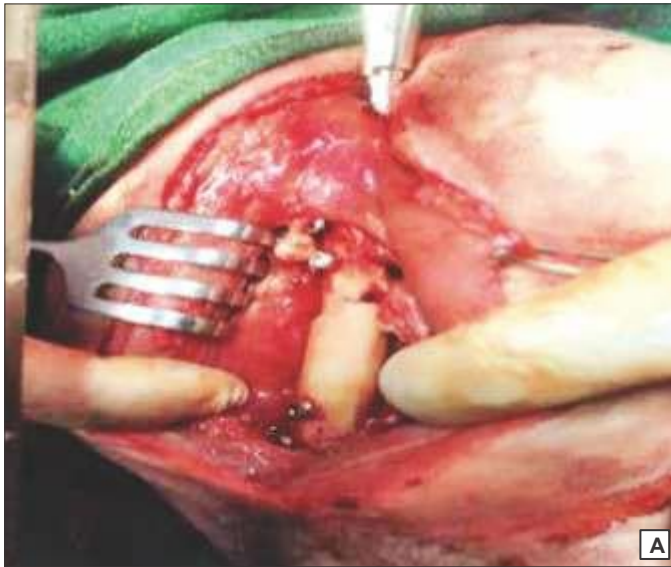


Fig. 1. Internal fixation of fracture using, A) Interlocking nailing, B) Application of  $\beta$ -TCP

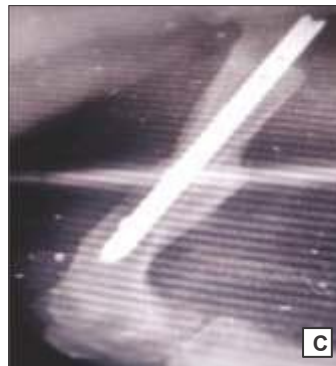


Fig. 2. Radiographs showing fracture healing in group I (upper row) and group II (lower row) Immediately after surgery, B. On 15<sup>th</sup> day, C. On 30<sup>th</sup> day, D. On 60<sup>th</sup> day)







Fig. 3. Weight bearing while standing in group I (upper row) and group II (lower row) (A. On 15<sup>th</sup> day, B. On 30<sup>th</sup> day, C. On 60<sup>th</sup> day)

effectiveness of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) in promoting bone healing. At various time intervals, the bone formation scores (BFS) in group II were not significantly higher than those in group I (Table 6). Similar to this, group II's bone union scores (BUS) were not significantly higher than group I's (Table 7). Group II has reached the stage of union earlier than Group I (Table 8 and Fig. 2). The administration of the osteoconductive substance,  $\beta$ -TCP, at the fracture site has resulted in an

**Table 5. Functional limb usage assessment (Fox *et al.*, 1995)**

Grade	Description
Functional limb usage	
Excellent	Weight bearing without lameness
Good	Slight Lameness
Fair	Slight to moderate lameness principally after exercise
Poor	Intermittent or consistent non-weight bearing lameness

**Table 6. Bone formation scores at different time intervals in both the groups (Mean $\pm$ S.E.)**

Time interval	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
Group I	0.00 <sup>a</sup> $\pm$ 0.00	0.67 <sup>b</sup> $\pm$ 0.21	1.67 <sup>c</sup> $\pm$ 0.21	3.00 <sup>d</sup> $\pm$ 0.26
Group II	0.00 <sup>a</sup> $\pm$ 0.00	0.83 <sup>b</sup> $\pm$ 0.31	2.00 <sup>c</sup> $\pm$ 0.26	3.33 <sup>d</sup> $\pm$ 0.33

(Means with different superscripts (a, b)/ (A, B) varies significantly ( $p < 0.05$ ) within group/ between the group)

**Table 7. Bone union scores at different intervals in both the groups (Mean $\pm$ S.E.)**

Time interval	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
Group I	0.00 <sup>a</sup> $\pm$ 0.00	0.67 <sup>ab</sup> $\pm$ 0.42	2.00 <sup>cd</sup> $\pm$ 0.00	0.67 <sup>d</sup> $\pm$ 0.42
Group II	0.00 <sup>a</sup> $\pm$ 0.00	0.67 <sup>b</sup> $\pm$ 0.42	2.00 <sup>abc</sup> $\pm$ 0.00	3.00 <sup>e</sup> $\pm$ 0.45

(Means with different superscripts (a, b) varies significantly ( $p < 0.05$ ) within group)

**Table 8. Stages of bone union at different time intervals in group I and group II**

Group I	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
IA	Not Achieved	Not Achieved	Uncertain	Achieved
IB	Not Achieved	Not Achieved	Uncertain	Uncertain
IC	Not Achieved	Not Achieved	Not Achieved	Uncertain
ID	Not Achieved	Uncertain	Uncertain	Achieved
IE	Not Achieved	Not Achieved	Uncertain	Achieved
IF	Not Achieved	Not Achieved	Uncertain	Uncertain
<b>Group II</b>				
IIA	Not Achieved	Uncertain	Achieved	Achieved
IIB	Not Achieved	Not Achieved	Uncertain	Uncertain
IIC	Not Achieved	Uncertain	Uncertain	Achieved
IID	Not Achieved	Not Achieved	Not Achieved	Uncertain
IIE	Not Achieved	Uncertain	Achieved	Achieved
IIF	Not Achieved	Uncertain	Achieved	Achieved

increase in the rate of bone formation, which has led to an increase in the mean bone union scores in group II. The osteoconductive properties of  $\beta$ -TCP were also described by Goel *et al.* (2013) and Preethi *et al.* (2021), demonstrating that it was a secure and efficient therapeutic choice for

fracture repair in significant osteo-periosteal abnormalities.

The mean weight bearing score when standing (Table 9) gradually increased over time in both groups, moving from  $0.00 \pm 0.00$  on day 0 to  $2.67 \pm 0.21$  on day 60<sup>th</sup> in group I and  $0.00 \pm 0.00$  to  $2.83 \pm 0.17$  on day 60<sup>th</sup> in group

**Table 9. Weight bearing scores while standing at different time intervals in both the groups (Mean  $\pm$  S.E.)**

Group	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
Group I	0.00 <sup>a</sup> $\pm$ 0.00	0.67 <sup>b</sup> $\pm$ 0.21	1.50 <sup>c</sup> $\pm$ 0.22	2.67 <sup>d</sup> $\pm$ 0.21
Group II	0.00 <sup>a</sup> $\pm$ 0.00	0.83 <sup>b</sup> $\pm$ 0.17	1.67 <sup>c</sup> $\pm$ 0.21	2.83 <sup>d</sup> $\pm$ 0.17

(Means with different superscripts (a, b)/ (A, B) varies significantly ( $p < 0.05$ ) within group/between group, respectively)

**Table 10. Weight bearing scores while walking at different time intervals in both the groups (Mean  $\pm$  S.E.)**

Group	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
Group I	0.00 <sup>a</sup> $\pm$ 0.00	0.83 <sup>a</sup> $\pm$ 0.40	2.00 <sup>b</sup> $\pm$ 0.52	3.17 <sup>c</sup> $\pm$ 0.48
Group II	0.00 <sup>a</sup> $\pm$ 0.00	1.50 <sup>b</sup> $\pm$ 0.43	2.50 <sup>b</sup> $\pm$ 0.43	3.50 <sup>c</sup> $\pm$ 0.34

(Means with different superscripts (a, b) varies significantly ( $p < 0.05$ ) within group)

II, respectively (Fig. 3). For the entire post-operative period, group II's mean weight bearing scores were higher. Also, on the day of the presentation, all animals in both groups displayed non-weight bearing lameness. Because a fracture results in pain in the affected area from inflammation and injury to the surrounding muscles, animals often lift the injured limb off the ground (Gupta, 2015). On the fifteenth day, group II's weight bearing scores non-significantly increased in comparison to group I. In addition, all of the animals in group II were observed contacting the toe or paw of the ground, but most of the animals in group I were carrying the limb off the ground. On the 30<sup>th</sup> day, the majority of the animals were discovered touching a toe or paw on the ground, showing that group I's weight bearing score had improved, whereas in group II, majority of the animals only began fully bearing weight on the injured limb on the 30<sup>th</sup> day. On the 60<sup>th</sup> postoperative day, animals from both groups displayed full weight bearing.

Similarly, the weight bearing scores while walking (Table 10) was non-significantly higher in group II as compared to group I at different intervals. Singh *et al.* (2020) noted related results as well. The early and better fracture healing in dogs of group II, which was supported by radiographic scores and was probably connected to local administration of  $\beta$ -TCP at the fracture site, was the cause of the improvement in the weight bearing scores (when standing and walking). The group that received osseomold (DMB and Calcium sulphate hemihydrate) and autogenous cancellous bone graft at the fracture site had the lowest mean lameness scores, which was a sign of early fracture healing, according to Kumar (2020).

In group I, there was one case where the functional use of the limb was excellent, three cases where it was good, one case where it was fair, and one case where it was bad. In group II, three examples were excellent, two were good, and one was fair. The timing of the recovery of limb functions with complete range of motion in group II

animals who had early callus development and weight bearing is consistent with this finding. Singh *et al.* (2020) also reported similar results.

Due to its osteoconductive properties and improved *in vivo* degradation,  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) has emerged as a viable material for applications involving bone regeneration. Through controlling osteogenic processes like the differentiation of mesenchymal stem cells into osteoblasts, the development of new blood vessels, the release of angiogenic growth factors, and blood clot formation,  $\beta$ -TCP promote bone regeneration (Lu *et al.*, 2021). In the group of animals receiving  $\beta$ -TCP treatment, the weight bearing scores, bone union scores, and functional limb usage were all improved. Thus,  $\beta$ -TCP helps dogs heal faster. As a result, it is determined that interlocking nailing in  $\beta$ -TCP is superior to interlocking nailing alone for the treatment of long bone fractures in dogs.

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