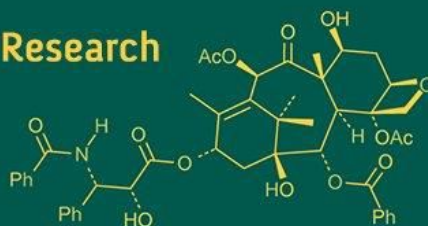


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Evaluation of biochemical markers for femoral or tibial fracture repair using LCP augmented with BHA granules in dogs

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Abstract

This clinical study evaluated biochemical markers of fracture healing in dogs treated with LCP with or without BHA granules. Twelve dogs with femoral or tibial fractures were randomly assigned to two groups: Group I treated with LCP along with BHA granules and Group II treated with LCP alone. Serum alkaline phosphatase, serum calcium and serum phosphorus levels were measured pre-operatively and on 0, 15, 30, 45 and 60 post-operative days. Serum ALP levels increased post-operatively in both groups. However, the peak values were earlier in group II on day 15 when compared to group I on day 30, indicating prolonged osteoblastic activity and enhanced callus mineralization with BHA, though all values remained within physiological limits. The higher ALP values could be attributed to the damaged periosteum during fracture and at the time of the bone plating procedure. Serum calcium and phosphorus levels showed transient early increases followed by declines and later normalization, reflecting osteoclastic resorption, mineral deposition, and bone remodeling during fracture healing. Excess calcium deposition at the fracture site likely reduced serum calcium early, followed by an increase during later bone remodeling. Both LCP augmented with BHA granules and LCP alone ensured effective fracture stabilization and good biochemical healing responses, emphasizing the significance of biochemical markers in evaluating fracture healing.

Keywords: Canine fractures, bovine hydroxyapatite (BHA), LCP, serum alkaline phosphatase, serum calcium, serum phosphorus

Introduction

Fractures are disruptions in the continuity of hard connective tissue caused by excessive forces that compromise bone integrity. In dogs, they commonly result from trauma and may significantly affect health and mobility. Such injuries are typically associated with mechanical stresses including bending, compression, torsion, and shearing (Perren *et al.*, 2002) [9]. The primary objectives of fracture treatment are to restore full limb function and enable early ambulation. For unstable fractures, the preferred method is open reduction and internal fixation (ORIF). Common fixation techniques include dynamic compression plating (DCP), locking compression plates (LCP), intramedullary interlocking nails, intramedullary pinning, cross pinning, and external skeletal fixation (Dueland *et al.*, 1999) [3]. Fractures with bone loss require bone grafts to fill defects and fixation devices for stability; bone grafting (autograft, allograft, xenograft) is routine to enhance healing, especially in complex long bone fractures (Sen *et al.*, 2018) [10]. Autografts remain the gold standard due to osteogenic, osteoinductive, and osteoconductive properties without immunogenic reactions, while allografts are limited by disease transmission, poor fusion, and processing challenges (Khan *et al.*, 2008) [7].

Bone tissue engineering and biomaterials (polymers, bio-ceramics, magnesium-based materials, metallic alloys) support faster healing and recovery; bio-ceramics like BHA, dicalcium phosphate, and tricalcium phosphate show promising results (Zhang *et al.*, 2011) [13]. However, limited information exists regarding the biochemical changes associated with these LCP along with BHA granules as xenograft. The present study aims to evaluate serum marker dynamics in dogs treated with LCP along with BHA granules and LCP alone, thereby establishing correlations between biochemical fluctuations and the progression of fracture healing.

Materials and Methods

The clinical investigation was carried out in the Department of Veterinary Surgery and Radiology, Veterinary College, Bidar, with the objective of evaluating the effectiveness of LCP along with BHA for managing long bone fractures in dogs. A total of 12 canine patients with femoral or tibial

fractures were randomly allocated into two groups of six (Table 1). Fracture stabilization in Group I was achieved using LCP along with BHA, whereas Group II received fixation with LCP alone. All surgeries were performed through open reduction and internal fixation (ORIF) technique.

Table 1: Design of study for the repair of femoral and tibial fracture in dogs

Groups	No. of dogs	Method and implant used for fracture repair in dogs
I	06	Long bone fracture repair by using LCP fixation along with BHA as xenograft at the site of fracture
II	06	Long bone fracture repair by using LCP fixation alone

Clinical observations, including swelling, crepitus, pain and absence of weight-bearing on the affected limb, were documented (Fossum, 2013) [4]. Radiographic assessment was performed using medio-lateral and cranio-caudal views to determine fracture type and implant dimensions. The required screw length and plate size were estimated from measurements taken on the contra-lateral limb (Guiot and Dejardin, 2012) [5].

Prior to surgery, animals were fasted for 12 hours and water was withheld for 6 hours. The operative site was aseptically prepared. Ceftriaxone (25 mg/kg IV) and Meloxicam (0.2 mg/kg IV) were administered 30 minutes before anaesthetic induction. All dogs Premedicated with atropine sulphate (0.045 mg/kg IM) followed by xylazine hydrochloride (1 mg/kg IM). Anaesthesia was induced with propofol (3 mg/kg IV to effect) and general anaesthesia maintained using isoflurane (2-2.5%) in oxygen. Endotracheal intubation was performed with appropriately sized tubes.

Dogs were positioned in lateral recumbency. Femoral fractures were exposed through a cranio-lateral incision, involving dissection of the tensor fascia lata, biceps femoris, and vastus lateralis muscles. Tibial fractures were approached medially, with careful preservation of neurovascular structures. Fracture fragments were anatomically aligned and stabilized using LCP plates with suitable screw configurations. In Group I after LCP fixation, one mL of autologous blood was drawn aseptically from the same animal during the intraoperative period. The autologous blood and BHA granules were mixed in a sterile petri plate in 1:1 ratio. The prepared composite was applied to the bone void using a sterile spatula. Surgical wound was closed in layers with polyglycolic acid for muscles and subcutaneous tissue and polyamide sutures for skin. Standard orthopaedic instruments, including bone holding forceps, drill sleeves, depth gauges and battery-powered drills were employed. Implants consisted of 2.7 mm and 3.5 mm LCP plates (6-11 holes) with locking screws respectively ranging from 14-26 mm.

Postoperative care included daily IV ceftriaxone (25 mg/kg) for seven days and Meloxicam (0.2 mg/kg) for three days. Dressings were changed every alternate day and sutures were removed on day 14. Controlled leash walking and passive range-of-motion exercises were recommended for 4-6 weeks. Clinical assessments were carried out on days 0, 15, 30, 45 and 60 to evaluate weight-bearing capacity and implant stability. Evaluation of biochemical markers such as serum alkaline phosphatase, calcium and phosphorus were measured preoperatively and on 0th, 15th, 30th, 45th and 60th post-operative day using a biochemical analyzer (ERBA-Mannheim, Germany). Intra-operative and postoperative complications were recorded. Statistical

analysis of biochemical data was performed using Student's *t*-test (Snedecor and Cochran, 1994) [12], while qualitative variables were assessed using a scorecard system. A *p*-value less than 0.05 was considered statistically significant.

Results

Serum ALP (IU/L)

The serum ALP levels (IU/L) recorded on the pre-operative and on 0th, 15th, 30th, 45th, and 60th post-operative days were 105.33±2.04, 108.67±1.71, 114.50±1.84, 129.00±1.15, 123.83±1.70, and 118.67±1.58, respectively. In Group I, the mean±SE values of serum ALP showed a non-significant increase on 0th post-operative day followed by significant increase ($p \leq 0.01$) on 15th, 30th, 45th and 60th post-operative days when compared to their pre-operative values.

The serum ALP levels (IU/L) recorded on the pre-operative and on 0th, 15th, 30th, 45th, and 60th post-operative days were 103±1.81, 109.5±2.19, 116.17±1.96, 112.67±1.93, 107.50±1.67, and 103.83±1.74, respectively. In Group II, the mean±SE values of serum ALP showed a significant increase ($p \leq 0.05$) on 0th post-operative day followed by significant increase ($p \leq 0.01$) on 15th, 30th, 45th and 60th post-operative days when compared to their pre-operative values. All recorded values in both the groups remained within the normal physiological range throughout the study period.

When compared between the groups, there was a significant difference ($p \leq 0.05$) in serum ALP levels on the 30th, 45th and 60th post-operative days.

Serum Calcium (mg/dL)

The Mean±SE serum calcium values recorded on the pre-operative and on 0th, 15th, 30th, 45th, and 60th post-operative days were 9.63±0.26, 9.70±0.20, 9.27±0.08, 9.12±0.34, 9.03±0.11 and 10.12±0.22, respectively. In Group I, serum calcium concentrations differed non-significantly on the 0th, 15th, 30th, 45th, and 60th post-operative days when compared to their pre-operative value.

The Mean±SE serum calcium values recorded on the pre-operative and on 0th, 15th, 30th, 45th, and 60th post-operative days were 9.32±0.15, 9.55±0.15, 9.30±0.18, 8.77±0.28, 8.95±0.17 and 9.07±0.16, respectively. In Group II, serum calcium concentrations differed non-significantly on the 0th, 15th, 30th, 45th, and 60th post-operative days when compared to their pre-operative value. All recorded values in both the groups remained within the normal physiological range throughout the study period.

Comparing both the groups, there was a significant difference ($p \leq 0.05$) in mean serum calcium concentration on the 60th post-operative day. All recorded values remained within the normal physiological range throughout the study period (Table 1).

Serum Phosphorus (mg/dL)

The serum phosphorus values recorded on the pre-operative and on 0th, 15th, 30th, 45th, and 60th post-operative days were 4.45±0.12, 4.57±0.12, 4.58±0.16, 4.70±0.06, 5.08±0.14 and 4.42±0.10, respectively. In Group I, serum phosphorus levels increased non significantly on 0th, 15th, 30th days and significantly ($p \leq 0.01$) on 45th post-operative day, followed by non-significant decrease on 60th day when compared to their pre-operative values.

The serum phosphorus values recorded on the pre-operative and on 0th, 15th, 30th, 45th, and 60th post-operative days were 4.43±0.20, 4.55±0.13, 4.82±0.23, 4.68±0.18, 4.43±0.20 and 4.32±0.21, respectively. In Group II, serum phosphorus

levels increased non significantly on 0th, 15th and 30th days followed by non-significant decrease on 45th and 60th day when compared to their pre-operative values. All recorded values in both the groups remained within the normal physiological range throughout the study period.

When comparing groups, a statistically significant difference ($p \leq 0.05$) was observed on the 45th post-operative day. All measured values remained within the normal physiological range throughout the study period.

Table 2 illustrates the variations in mean±SE values of serum alkaline phosphatase, serum calcium and serum phosphorus across different time intervals.

Table 2: Mean±SE of biochemical parameters at different intervals in dogs of Group I and Group II

Biochemical parameter	Groups	Pre-operative	0 th day	15 th day	30 th day	45 th day	60 th day
Serum alkaline phosphatase (ALP)	I	105.33±2.04	108.67±1.71	114.50±1.84**	129.00±1.70***a	123.83±1.70***a	118.67±1.58***a
	II	103.00±1.81	109.50±2.19*	116.17±1.96**	112.67±1.93**b	107.50±1.67**b	103.83±1.74**b
Serum calcium (Ca)	I	9.63±0.26	9.20±0.20	9.27±0.08	9.12±0.34	9.03±0.11	10.12±0.22 ^a
	II	9.32±0.15	9.55±0.15	9.30±0.18	8.77±0.28	8.954±0.17	9.07±0.16 ^b
Serum phosphorous (P)	I	4.45±0.12	4.15±0.12	4.58±0.16	4.70±0.06	5.08±0.14***a	4.42±0.10
	II	4.43±0.20	4.55±0.13	4.82±0.23	4.68±0.18	4.43±0.20 ^b	4.32±0.21

- Means bearing superscript ** differs significantly ($p \leq 0.01$) with in group from pre-operative day
- Means bearing superscript * differs significantly ($p \leq 0.05$) with in group from pre-operative day
- Means bearing superscript ^{a, b} differs significantly ($p \leq 0.05$) between the group at corresponding intervals

Discussion

Serum ALP (IU/L)

Group I showed a steady rise up to the 30th post-operative day followed by decline till day 60, while Group II peaked earlier on the 15th day and then declined. Serum levels of ALP were higher in relation with osteoblastic activity at the fracture site (Komnenou *et al.*, 2005)^[8]. In the current study, ALP values showed gradual increase during the preoperative and on 0th, 15th and 30th postoperative days. The higher ALP values could be attributed to the damaged periosteum during fracture and at the time of the bone plating procedure. Based on these results, it appeared that callus development was evident on the 30th postoperative day, when osteoblastic activity peaked which was in agreement with that of Chaudhari *et al.* (2000)^[11].

The values of ALP were found elevated in Group-I than group II on the 15th, 30th, 45th and 60th postoperative days indicating more osteoblastic activity at the site of fracture in these dogs. The use of BHA granules at the fracture site might have encouraged this augmented activity as opined by Chaurasia *et al.* (2019)^[2].

Serum Calcium (mg/dL)

Both groups showed an initial rise on day 0, followed by gradual decline up to day 45 in Group I and day 30 in Group II, and a subsequent increase till day 60. Early increase was attributed to osteoclastic activity, while later rise was linked to bone remodelling Singh *et al.* (2017)^[11]. A significant intergroup difference was noted on day 60, possibly due to delayed remodelling in Group I with BHA, however all values remained within normal range.

The deposition of excess calcium at the fracture site might have led to decreased serum calcium levels during the early stages of bone healing. Bone remodelling might have contributed to a subsequent increase in the levels during the later stages of fracture healing as explained by Chaurasia *et al.* (2019)^[2].

Serum Phosphorus (mg/dL)

Serum phosphorus increased up to day 45 in Group I and day 15 in Group II, followed by gradual decline till day 60, with peak values on respective days. Elevated levels were linked to enhanced healing, calcium deposition, and callus ossification, especially in Group I due to BHA Hegde *et al.* (2007)^[6]. A significant intergroup difference was observed on day 45, with all values within physiological limits.

The high levels of extracellular concentrations of phosphorus resulted in the deposition of calcium at the site of fracture, thereby facilitating the ossification of the callus as reported by Komnenou *et al.*, (2005)^[8].

Conclusion

The sustained elevation and delayed peak of ALP in Group I indicate prolonged osteoblastic activity and enhanced callus formation, suggesting that BHA promotes extended bone formation compared to LCP alone. The earlier peak and faster decline in Group II reflect a relatively quicker transition from callus formation to remodelling, whereas Group I shows a more gradual healing progression. The biphasic pattern observed in both groups confirms normal bone healing physiology involving early osteoclastic resorption followed by mineralization and remodelling. The delayed increase in serum calcium in Group I suggests prolonged remodelling activity, likely influenced by the osteoconductive effect of BHA. Elevated phosphorus levels during early to mid-healing phases indicate active mineral deposition and callus ossification, with Group I showing a prolonged elevation consistent with sustained healing activity. Significant differences at specific time points of days 30, 45, and 60, highlight that BHA modifies the rate and duration of biochemical responses without altering their physiological pattern. All biochemical parameters remained within normal reference ranges in both groups, confirming that the use of BHA was biochemically safe. The biochemical profiles suggest that BHA enhances and prolongs the bone healing cascade, particularly in terms of

osteoblastic activity and mineralization, without causing metabolic imbalance.

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