Haemato-biochemical parameters in healing of Femur diaphyseal fracture stabilized with locking compression plate and autologous cancellous bone graft in canines

G. Narendra Bhargav[1*], R. Mahesh[2], Ch. Mallikarjuna Rao[3], K. Suresh[4] and K. Rambabu[5]

[1,3,5]Department of Veterinary Surgery and Radiology, [4]Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram; Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh; [2]Veterinary Clinical Complex, College of Veterinary Science, Garividi, Andhra Pradesh.
*Corresponding author- Email: bhargavgora@gmail.com

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Abstract

The present study evaluated the haemato-biochemical parameters in dogs undergoing stabilization of femur diaphyseal fractures using locking compression plate alone (n=6) and locking compression plate with autologous cancellous bone graft (n=6. Hematological parameters, including haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte counts were assessed preoperatively and postoperatively on 1st, 21st, 42nd and 60th days. Biochemical parameters including blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium and phosphorus were also analyzed. Both groups exhibited a significant decrease in Hb, TEC and PCV on the 1st postoperative day followed by gradual recovery by the 21st day with no significant differences between groups. TLC and neutrophil percentages decreased significantly by the 21st day, while lymphocyte percentages increased. Eosinophil and monocyte counts remained stable and within normal ranges. Biochemical analysis revealed stable BUN and creatinine levels. ALP levels increased significantly postoperatively peaking at the 21st day, with group II showing higher ALP levels than group I. Calcium levels were significantly higher in group II from the 21st day onward, while phosphorus levels remained stable, with group II showing a slight increase at the 42nd day. The addition of autologous cancellous bone graft in group II influenced calcium metabolism and ALP levels, suggesting potential bone healing activity.

Key words: Alkaline phosphatase, femur, locking compression plate, autologous cancellous bone graft.

Introduction

A fracture is a break in continuity of hard tissues like bone, cartilage. Trauma is the most common cause of fractures in small animals and can occur due to bending, torsional, shearing and compression forces, eventually resulting in oblique, wedge fragment, spiral or comminuted fractures (Usadadiya et al., 2020). Bone healing is primarily monitored through physical examinations and serial radiographic imaging of the fracture site. However, distinguishing between delayed union and nonunion can be challenging without advanced imaging techniques. In such cases, serum biochemical markers of bone formation, such as alkaline phosphatase (ALP) activity, can provide valuable clinical insights into the progression of healing (Komnenou et al., 2005). The primary objective of fracture treatment is to achieve healing with proper bone alignment. Radiography remains the standard method for assessing fracture healing, while hematobiochemical parameters play a significant role in evaluating both patient health and fracture recovery (Phaneendra et al., 2016; Patil et al., 2017; Chaurasia et al., 2019). Biochemical markers of bone are categorized into bone resorption markers and bone formation markers. Bone resorption markers are associated with osteoclast activity whereas bone formation marker such as bone-specific alkaline phosphatase is produced by osteoblasts (Delmas 1995). Elevated levels of alkaline phosphatase can be linked to the proliferation of osteogenic cells and the significant contribution from the periosteum of damaged bone, which is a rich source of serum alkaline phosphatase (Belic et al., 2010). Bone grafts and their substitutes enhance fracture healing through osteogenesis, osteoinduction and osteoconduction (Johnson 2013). The use of autologous cancellous bone graft has been shown to improve fracture healing during the phase of recovery (Fesseha 2020, Pecin et al., 2021 and Cho et al., 2023).

Materials and Methods

The study evaluated the efficacy of locking compression plates (LCP) with and without autologous cancellous bone grafts in stabilizing femur diaphyseal fractures in dogs. Clinical cases of femur diaphyseal fractures presented to the Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram and SVVU Super Specialty Veterinary Hospital, Visakhapatnam from December 2023 to November 2024 were included. Dogs with non-weight bearing lameness underwent orthopaedic and neurological examinations and clinical signs such as localized edema, pain and crepitus were recorded. Radiographic evaluations (craniocaudal and mediolateral views) were performed using an Adonis 100 mA X-ray unit with sedation using Butorphanol tartrate (0.2 mg/kg IM). Temporary stabilization was achieved with over the hip bandage and preoperative care included Cefotaxime (50 mg/kg IM) and Meloxicam (0.2 mg/kg IM). Twelve dogs with closed femur diaphyseal fractures were divided into two groups: Group I (LCP alone) and Group II (LCP with autologous cancellous bone graft). Preoperative preparation included fasting (12 hours) and withholding water (6 hours). Anesthesia was induced with Butorphanol tartrate (0.2 mg/kg IM), Atropine sulphate (0.04 mg/kg SC), Ketamine hydrochloride (5 mg/kg IV) and Midazolam (0.2 mg/kg IV) and maintained with Isoflurane and oxygen. The surgical site was aseptically prepared using chlorhexidine (1.5%), povidone-iodine (5%) and isopropyl alcohol (70%). Autologous cancellous bone grafts were harvested from the humerus and packed at the fracture site in Group II. Postoperative care included Cefotaxime (50 mg/kg IM for 5 days), Meloxicam (0.2 mg/kg IM for 3 days) and oral calcium syrup. Fracture healing was assessed using lameness grading, clinical outcomes and radiographic evaluations. Hematological parameters (haemoglobin, packed cell volume, total erythrocyte count and total and differential leukocyte count) were analyzed using an autoanalyzer, with blood samples collected in EDTA vials preoperatively and on the 1st, 21st, 42nd and 60th postoperative days. Biochemical parameters (BUN, creatinine, alkaline phosphatase, aspartate aminotransferase, calcium and phosphorus) were estimated using an automatic biochemical analyzer, with serum samples collected in clot activator vials at the same intervals. Statistical analysis was performed using SPSS Statistics version 20, with independent sample t-tests and ANOVA with Tukey HSD post hoc test.

Results

Hematological parameters

The details of various hematological parameters estimated were given in table 1 and 2.

Haemoglobin (g/dL)

In group I, the haemoglobin levels decreased significantly on the 1st postoperative day compared to the preoperative values but increased significantly thereafter reaching near preoperative levels by the 21st POD. Similarly in group II, there was a significant decrease on the 1st POD followed by a gradual and significant recovery by the 21st POD. No significant differences were observed between the groups at different time intervals. However, all values remained within the normal physiological range throughout the study.

Table 1. The mean+ SE values of hematological parameters in groups I & II during different periodic interval

Parameter	Group	Preoperative	1st POD	21st POD	42nd POD	60th POD	Total mean
Hb (g/dL)	I	12.66±0.49^a	11.90±0.55^b	13.17±0.39^a	13.22±0.18^a	13.10±0.14^a	**12.81±0.56**
	II	13.23±0.22^a	12.97±0.12^b	13.81±0.16^a	13.15±0.14^a	13.40±0.17^a	**13.31±0.32**
TEC (Millions/µL)	I	6.23±0.39^a	5.89±0.20^b	6.58±0.19^a	6.61±0.09^a	6.55±0.07^a	**6.37±0.31**
	II	6.62±0.11^a	6.63±0.21^a	6.37±0.48^a	6.28±0.13^b	6.23±0.39^b	**6.43±0.19**
PCV (%)	I	37.67±0.81^a	35.37±1.22^b	39.50±1.16^a	39.65±0.55^a	39.30±0.42^a	**38.30±1.82**
	II	39.70±0.65^a	38.90±0.36^b	39.55±0.48^a	37.67±0.81^b	37.67±0.81^b	**38.70±0.99**
TLC (Thousand/µL)	I	14.53±0.79^a	14.38±0.83^a	11.68±0.55^b	11.65±0.30^b	11.68±0.29^b	**12.78±1.53**
	II	14.25±0.81^a	14.07±0.81^a	12.27±0.72^b	12.50±0.68^b	11.97±0.69^b	**12.81±1.09**

Normal Physiological range of Hemoglobin in dogs is 12-19 g/dL, TEC in dogs is 5-7.9 Millions/µL, PCV in dogs is (35-55) %, TLC in dogs is 5-14.1 Thousands/µL; Means bearing different superscripts within a row (a, b..) and within a column (1, 2) differs significantly (P<0.05)
POD- Post operative day

Table 2. The mean+ SE values of differential leukocyte count in groups I & II during different periodic interval

White Blood Cells	Group	Preoperative	1st POD	21st POD	42nd POD	60th POD	Total mean
Neutrophils (%)	**I**	81.33±1.37^a	81.33±1.37^a	68.67±1.37^b	65.50±1.76^c	68.67±1.37^b	**73.10±1.50**
	II	80.67±1.03^a	81.33±1.37^a	69.67±1.51^b	63.67±2.25^c	59.17±2.32^d	**70.02±1.94**
Lymphocytes (%)	**I**	15.83±0.75^a	15.83±0.75^a	28.33±1.21^b	31.33±1.21^b	28.33±1.21^b	**23.92±1.04**
	II	16.50±0.84^a	15.83±0.75^a	27.67±1.75^b	33.17±2.04^a	37.67±2.73^a	**26.83±1.81**
Eosinophils (%)	**I**	1.67±0.82^a	1.67±0.82^a	1.83±0.98^a	1.83±0.41^a	1.83±0.98^a	**1.73±0.81**
	II	1.83±0.98^a	1.67±0.82^a	1.67±0.82^a	2.00±0.63^a	2.00±0.89^a	**1.83±0.83**
Monocytes (%)	**I**	1.17±0.41^a	1.17±0.41^a	1.33±0.52^b	1.17±0.41^a	1.17±0.41^a	**1.20±0.41**
	II	1.17±0.41^a	1.17±0.41^a	1.17±0.41^a	1.33±0.52^b	1.17±0.41^a	**1.20±0.41**

Normal Physiological range of neutrophil count in dogs 58-85%, Lymphocytes (8-21%), Eosinophils (0-9%) and Monocytes (0-9%)
Means bearing different superscripts within a row (a, b..) and within a column (1, 2) differs significantly (P<0.05)
POD- Post operative day

Total Erythrocyte Count (TEC) (Millions/µL)

In group I, TEC showed a significant decrease on the 1st postoperative day compared to the preoperative values but increased significantly by the 21st POD, maintaining near preoperative levels thereafter. In group II, TEC values remained relatively stable from the preoperative period to the 1st POD but showed a gradual and slight decline thereafter, particularly by the 42nd and 60th PODs. No significant differences were observed between the groups at different time intervals. All values remained within the normal physiological range throughout the study.

Packed Cell Volume (PCV) (%)

In both groups, PCV values non significantly decreased on the 1st POD ($P < 0.05$) compared to the preoperative levels. In Group I, PCV levels then increased non significantly by the 21st POD and remained stable through the 42nd and 60th PODs. In contrast, Group II showed a more gradual recovery, with non significant decreases observed at the 42nd and 60th PODs compared to the preoperative values ($P < 0.05$). The PCV levels in Group I were consistently higher than those in Group II after the 21st POD. Despite these fluctuations, all PCV values remained within the normal physiological range throughout the study.

Total Leukocyte Count (TLC) (Thousand/µL)

In both groups, TLC values remained relatively stable from the preoperative period to the 1st POD. However, a significant decrease was observed by the 21st POD, which remained consistent through the 42nd and 60th Pods. No significant differences were observed between the groups at any time interval. All TLC values remained within the normal physiological range throughout the study.

Differential Leucocyte count

The details of differential leucocyte values were furnished in table no 2.

Neutrophils (%)

In both groups, neutrophil percentages were highest during the preoperative period and on the 1st (POD). A significant decrease was observed by the 21st POD, followed by further significant reductions by the 42nd POD. By the 60th POD, values in both groups showed a slight recovery. Significant differences were observed between the time intervals within each group, but no significant differences were noted between groups at corresponding time intervals. All neutrophil percentages remained within the normal physiological range throughout the study.

Lymphocytes (%)

In both groups, lymphocyte percentages remained stable from the preoperative period to the 1st POD. A significant increase was observed by the 21st POD, followed by further increases on the 42nd POD. By the 60th

POD, group I showed stabilization in lymphocyte percentages, whereas group II demonstrated a continued and significant increase. While significant changes were observed over time within each group, no significant differences were noted between groups at corresponding time intervals. All lymphocyte percentages remained within the normal physiological range throughout the study.

Eosinophils (%)

Throughout the study, eosinophil counts remained within the normal physiological range for both groups at all time intervals. Moreover there were no significant differences observed between the groups.

Monocytes (%)

Monocyte counts stayed within the normal physiological range in both groups across all time points in this study. Additionally no significant differences were noted between the groups at any time interval.

Biochemical studies

The details of various serum biochemical parameters estimated in the present study were given in table 3.

Table 3. The mean+ SE values of serum biochemical parameters in groups I & II during different periodic interval

Parameter	Group	Preoperative	1st POD	21st POD	42nd POD	60th POD	Total Mean ± SE
BUN (mg/dL)	I	17.37±0.48^{a}	17.22±0.61^{a}	17.37±0.48^{a}	17.44±0.61^{a}	17.37±0.48^{a}	**17.35±0.48**
	II	16.93±3.57^{a}	17.02±3.70^{a}	16.93±3.65^{a}	16.85±3.65^{a}	17.18±3.37^{a}	**16.98±3.58**
Creatinine (mg/dL)	I	1.00±0.24^{a}	0.97±0.20^{a}	0.98±0.22^{a}	1.05±0.22^{a}	1.03±0.22^{a}	**1.01±0.22**
	II	1.07±0.23^{a}	1.05±0.22^{a}	1.10±0.14^{a}	1.08±0.12^{a}	1.12±0.15^{a}	**1.08±0.15**
ALP (units/I)	I	74.00±14.87^{a}	117.83±17.98^{b}	145.50±13.19^{c1}	139.30±4.46^{d1}	124.60±1.92^{c}	**113.7±4.30^{1}**
	II	69.33±17.17^{a}	121.33±18.77^{b}	210.33±20.69^{d2}	171.67±21.49^{e2}	141.33±24.93^{c}	**152.9±9.83^{2}**
AST (units/I)	I	31.67±3.01^{a}	32.00±2.61^{a}	31.17±2.79^{a}	31.00±2.19^{a}	32.00±2.61^{a}	**31.77±2.64**
	II	32.67±8.29^{a}	33.33±8.33^{a}	33.17±8.61^{a}	33.50±7.84^{a}	32.83±8.13^{a}	**32.90±8.22**
Calcium (mg/dL)	I	9.50±0.57^{a}	9.62±0.41^{a}	9.43±0.53^{a1}	9.38±0.45^{a1}	9.42±0.50^{a}	**9.57±0.52**
	II	9.70±0.33^{a}	9.82±0.35^{a}	10.57±0.42^{b}	10.32±0.44^{b}	10.12±0.32^{b}	**10.50±0.40**
Phosphorus (mg/dL)	I	4.12±0.45^{a}	4.30±0.44^{a}	3.88±0.33^{a1}	3.77±0.45^{a1}	3.85±0.45^{a}	**3.88±0.44**
	II	4.30±0.46^{a}	4.38±0.44^{a}	4.70±0.33^{a}	4.87±0.23^{b}	4.83±0.24^{a}	**4.63±0.35**

Normal Physiological range of serum Blood Urea Nitrogen is 10–28 mg/dL, Creatinine is 0.5-1.5 mg/dL, Alkaline Phosphatase in dogs is 1-114 units/I, Aspartate amino transferase is 23–66 units/I, Calcium is 9.1-11.7 mg/dL Phosphorus is 2.9-5.3mg/dL

Means bearing different superscripts within a row (a, b..) and within a column (1, 2) differs significantly (P<0.05)

POD- Post operative day

Blood Urea Nitrogen (BUN) (mg/dL)

In both groups, BUN levels remained relatively stable from the preoperative period through the 60th POD, with no significant variations observed at any time point. Additionally, no significant differences were noted between Group I and Group II at any interval, indicating consistent and comparable BUN levels across both groups. All values remained within the normal physiological range throughout the study, suggesting stable renal function in both groups.

Creatinine (mg/dL)

Creatinine levels remained relatively stable across all time points for both groups, indicating stable kidney function throughout the study period. No significant variations or differences were noted between the groups, suggesting consistent renal health across the two groups.

Alkaline Phosphatase (units/Liter)

In both groups, the ALP levels significantly increased ($P < 0.05$) from the preoperative period to the 1st POD and peaked at the 21st POD. In Group I, the peak value was 145.50 ± 5.39 units/L, followed by a gradual and significant decrease ($P < 0.05$) through the 42nd POD and 60th POD. Similarly in Group II, the peak value was higher at 210.33 ± 8.45 units/L, with a subsequent significant decrease ($P < 0.05$) by the 60th POD. The difference between the groups from the 1st to the 42nd POD was significant ($P < 0.05$), with Group II consistently showing higher ALP levels compared to Group I. All observed values were above the normal physiological range during the study, with values returning to the normal range by the 60th POD. The difference in the total mean serum ALP concentration between the groups was also significant ($P < 0.05$).

Aspartate Aminotransferase (AST) (units/Liter)

In both groups, AST levels remained relatively stable throughout the study period, with no significant changes observed from the preoperative period through the 60th POD. The values for Group I and Group II were comparable at all time points, indicating consistent AST levels in both groups. All values remained within the normal physiological range for AST throughout the study suggesting stable liver function in both groups.

Calcium (mg/dL)

In both groups, calcium levels remained within the normal physiological range throughout the study, but significant differences between the two groups were observed by the 21st POD. Group II showed higher calcium levels compared to Group I, especially by the 21st, 42nd and 60th PODs ($P < 0.05$). These differences may suggest a potential alteration in calcium metabolism in Group II during the postoperative period.

Phosphorus (mg/dL)

In both groups, phosphorus levels remained relatively stable throughout the study period, with no significant differences between the groups at the preoperative, 1st POD and 21st POD. However, significant increases were observed in Group II at the 42nd POD (4.87 ± 0.09 mg/dL), compared to Group I, where levels remained stable. By the 60th POD, phosphorus levels in Group II decreased slightly but still remained higher than those in Group I. All values remained within the normal physiological range throughout the study.

Discussion

In a comparative study of fracture healing, significant changes in hematological and biochemical parameters were observed in two groups from the preoperative period to the 60th postoperative day (POD). Haemoglobin, packed cell volume (PCV) and total erythrocyte count (TEC) exhibited a notable decline from the preoperative period to the first POD followed by a gradual recovery. This trend aligns with findings by Lobo et al., (2013) and Chaurasia et al., (2017) who reported similar reductions due to surgical blood loss and mild haemodilution from intravenous fluids. The subsequent rise in these parameters likely reflects the initiation of erythropoiesis as noted by Lobo et al., (2013) and Chaurasia et al., (2019) indicating the body's effort to restore normal blood levels. However this contrasts with studies by Patil et al., (2017), Singh et al., (2017) and Reddy et al., (2021) which observed increased haemoglobin and TEC levels by the 60th POD.

Total leukocyte count (TLC) was elevated preoperatively, immediately post-surgery and gradually declining non significantly by the 60th POD. This pattern consistent with Patil et al., (2017) and Chaurasia et al., (2019) reflects an inflammatory response triggered by bone trauma and surgical intervention. Chaurasia et al., (2019) attributed preoperative TLC elevation to systemic inflammation while Patil et al., (2017) and Reddy et al., (2021) suggested contributions from corticosteroid release, stress, pain and surgical manipulation. The decline in TLC by the 60th POD indicates inflammation resolution, aligning with the natural progression of fracture healing.

Neutrophil counts followed a similar trend peaking preoperatively and immediately post-surgery then decreasing non significantly by the 60th POD. This neutrophilic leucocytosis a hallmark of early inflammation reflects neutrophils' role as the primary defence mechanism (Sastry, 1989). The gradual reduction noted by, Patil et al., (2017) and Chaurasia et al., (2019) signifies subsiding inflammation as tissue repair advances. Lymphocyte counts initially lower, increased non significantly by the 42nd POD and stabilized by the 60th POD which is consistent with Patil et al., (2017), Chaurasia et al., (2019) linked this relative lymphocytosis to inflammatory resolution. Eosinophil and monocyte counts remained within normal ranges indicating implant biocompatibility as supported by Patil et al., (2017) and Chaurasia et al., (2019).

Specific biochemical markers, including serum alkaline phosphatase (ALP), calcium and phosphorus levels can provide valuable insights into the healing process and help identify fractures at risk of progressing to non union (Komnenou et al., 2005 and Joshi et al., 2010). Elevated serum ALP levels, for instance are associated with increased osteoblastic activity at the fracture site reflecting ongoing bone formation (Komnenou et al., 2005).

In the present study, both groups exhibited lower ALP levels during the preoperative and immediate postoperative periods. However, a significant increase in ALP levels was observed at the 21st postoperative day followed by a notable decline by the 60th postoperative day ($p < 0.05$). This pattern is consistent with Komnenou et al., (2005) reporting a significant rise in ALP levels by the 10th postoperative day, which returned to baseline by the 30thday. The elevated ALP levels may be linked to periosteal damage occurring during the fracture and the bone plating procedure. These findings suggest that callus formation became evident around the 21st postoperative day coinciding with the peak of osteoblastic activity, a result consistent with the observations of Chaudhari et al., (2000).

A similar pattern in ALP levels was observed, with elevated values in the early postoperative period followed by a gradual reduction as the fracture healing progressed, as reported by Patil et al., (2017) and Gaddam et al., (2022). In this study, Group II exhibited higher ALP levels than Group I on the 21st and 42nd postoperative days indicating greater osteoblastic activity at the fracture site in these animals. This increased activity may be linked to the application of autologous cancellous bone grafts at the fracture site, a finding supported by Chaurasia et al., (2019).

Calcium levels in Group I remained normal throughout, aligning with Patil et al., (2017) and Gaddam et al., (2022). Group II however, showed significantly higher calcium levels on the 21st, 42nd and 60th PODs ($p < 0.05$) reflecting active bone remodelling as described by Chaurasia et al., (2019). Serum phosphorus levels in Group I stayed normal while Group II showed a significant increase by the 42nd POD suggesting enhanced healing through calcium deposition and callus ossification as per Komnenou et al., (2005). Blood urea nitrogen, creatinine and aspartate aminotransferase remained normal indicating no systemic complications.

Conclusion

In conclusion, both surgical methods were effective with stable hematological and biochemical parameters in both groups with significant postoperative changes in ALP and calcium levels. Group II showed higher ALP and calcium levels, suggesting enhanced bone metabolism. All values remained within normal ranges, indicating effective healing postoperatively.

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