The Pharma Innovation Journal 2023; SP-12(7): 1801-1803

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(7): 1801-1803

www.thepharmajournal.com
Received: 01-04-2023
Accepted: 08-05-2023

Ravi Kumar P
Assistant Professor, Department of Veterinary Surgery and Radiology, SVVU Super Speciality Veterinary Hospital, Affiliated to Sri Venkateswara Veterinary University, Visakhapatnam, Andhra Pradesh, India

Devi Prasad V
Professor and Head, Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

Sreenu M
The Associate Dean, College of Veterinary Science, Garividi, Andhra Pradesh, India

Venkata Naidu G
The Director of Extension, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Raju NKB
Professor, Department of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

Corresponding Author:
Ravi Kumar P
Assistant Professor, Department of Veterinary Surgery and Radiology, SVVU Super Speciality Veterinary Hospital, Affiliated to Sri Venkateswara Veterinary University, Visakhapatnam, Andhra Pradesh, India

Biochemical evaluation of the effect of biomaterials on long bone fractures stabilized with the application of positive profile end threaded stainless steel and titanium intramedullary pins in dogs

Ravi Kumar P, Devi Prasad V, Sreenu M, Venkata Naidu G and Raju NKB

Abstract
The current study was conducted on long bone fractures in dogs to assess the suitability of positive profile end threaded pins made of stainless steel and titanium, as well as the effect of biomaterials on fracture healing augmentation. Based on preoperative radiographic evaluation, the cases were randomly divided into four groups of six dogs. The fractures were stabilized with self-tapping positive profile end threaded intramedullary pins made of stainless steel in group I and titanium in groups II, III, and IV. The fracture gap was left unfilled in groups I and II, filled with Osseograft and Osseomold in group III, and filled with Osseograft and Autogenous Cancellous graft in group IV. On the preoperative day, the 7th, 15th, 30th, 60th, and 90th postoperative days, haematological and biochemical parameters were recorded and compared between the groups. The values of both haematological and biochemical parameters were within the normal range throughout all of the periodic intervals and appeared to fluctuate in relation to fracture healing in different groups.

Keywords: Long bone fractures, positive profile end threaded pins, alkaline phosphatase, serum calcium, serum phosphorus

Introduction
Fracture repair is distinguished by the creation of a new organic matrix (osteoid) and its subsequent mineralization, which eventually spans the space between two bony fragments (bridging callus) (Arnon 1998, Johnson and Watson 2000) [2, 10]. Age, fracture site, type of fixation (implant devices), stability, and blood supply all influence the time required for "typical" fracture healing. Delayed union or nonunion refers to the lengthening or failure of the healing process (Arnon, 1998; DeCamp 2003) [2, 8]. The healing process is monitored with physical and serial radiologic tests of the fracture site (Arnon, 1998; DeCamp 2003) [2, 8]. However, distinguishing a delayed union from a nonunion radiologically can be challenging (Kaderly 1993 and Betts 1995) [11, 3], and the use of more sophisticated imaging techniques, such as nuclear Scintigraphy, is currently limited in veterinary medicine (Betts 1995) [3]. Detecting specific biochemical indicators of bone formation in serum, such as alkaline phosphatase (ALP) activity, can thus be therapeutically relevant in monitoring the healing process (Nyman *et al* 1991) [15]. Other markers, such as serum calcium and phosphorus levels, may also be useful in evaluating improvement. The purpose of this study was to examine if serum ALP activity, calcium, and phosphorus levels were associated to fracture healing in dogs.

Materials and Methods
Based on preoperative radiographic evaluation, the cases were randomly divided into four groups of six dogs. The fractures were stabilized with self-tapping positive profile end threaded intramedullary pins made of stainless steel in group I and titanium in groups II, III, and IV. The fracture gap was left unfilled in groups I and II, filled with Osseograft and Osseomold in group III, and filled with Osseograft and Autogenous Cancellous graft in group IV. On the preoperative day, the 7th, 15th, 30th, 60th, and 90th postoperative days, the biochemical parameters were recorded and compared between the groups.

Results and Discussion

The mean ± SE values of the alkaline phosphatase, serum phosphorus and serum calcium of different groups at different periodic intervals were showed in the tables-1, 2 and 3 respectively. The alkaline phosphatase values showed a significant (p<0.05) rise from preoperative and immediate postoperative periods to the 30th postoperative day in all the groups and then found decreased. The values were above the normal physiological range in all the dogs at different periodic intervals, except in groups-III and IV, in which the values reached the normal physiological range by 90th postoperative day. Highest values were recorded on the 7th, 15th and 30th postoperative days and lowest values on the 90th postoperative day in group-IV, whereas, lowest values were recorded on the 7th, 15th and 30th postoperative day and highest values on 90th postoperative day in group-I.

The values of serum phosphorus were found to rise from pre and immediate postoperative days to the 30th postoperative day in groups-I and II; from pre and immediate postoperative day to the 15th postoperative day in groups-III and IV. All the groups showed lower values of serum phosphorus on the 90th postoperative day. The values differed significantly (p<0.05) among the groups on the 7th, 15th and 30th postoperative days with higher values in group-IV and lower values in group-I.

The values of serum calcium had decreased from pre and immediate postoperative days to the 30th postoperative day and then reached normal values by 90th postoperative day in all the groups. The values differed significantly (p<0.05) among different periodic intervals in group-III and IV. There was no significant difference in the values of serum calcium among different groups at various periodic intervals except on the 15th postoperative day on which significantly (p<0.05) higher values were recorded in group-I (8.98 ± 0.78). However, all the values were within the normal physiological range.

Fracture healing in Orthopaedic patients is usually monitored with the help of clinical and radiographic examination. However, these examination procedures could not distinguish the cases with delayed union from mal-union. (Kaderly, 1993 and Betts, 1995) [11, 3]. Bone marker like alkaline phosphatase was elevated in the serum, in relation to Osteoblastic activity that takes place at the fracture site (Leung *et al.*, 1993 and Komnenou *et al.*, 2005) [14, 12]. Hence, serum biomarker like alkaline phosphatase could be useful in evaluating the progress of healing process, especially in cases of delayed union and non-union (Allen *et al*., 1998, Komnenou *et al*., 2005 and Sousa *et al*., 2011) [1, 12, 17]. In the present study, higher alkaline phosphatase values were noticed in the dogs of all the four groups, during preoperative and immediate postoperative days, and these values further increased up to the 30th postoperative day and then started declining to normal values. These findings suggest that, the Osteoblastic activity was peak on the 30th postoperative day with obvious callus formation. Similar findings were also reported by Chaudhari *et al.* (2000) [6], Kulkarni (2000) [13], Bhalerao (2010) [4] and Chaurasia *et al.* (2019) [7]. The higher preoperative values could be attributed to increased release of alkaline phosphatase from the damaged periosteum as opined by Hegde *et al.* (2007) [9] and Patil *et al.* (2017) [16].

The values of alkaline phosphatase are higher in Group-III & IV dogs than the other two groups on the 7th, 15th and 30th postoperative days suggesting more Osteoblastic activity at the site of fracture in these dogs. This increased activity might have been favored by the application of biomaterials at the fracture site. Chaurasia *et al.* (2019) [7] opined that, implantation of Osteoinductive materials at fracture site, further increased the Osteoblastic activity. The normal values of alkaline phosphatase in Group-III & IV on 90th postoperative day suggested the completion of fracture healing. Bolger, (1975) [5] stated that, when callus formation ceased the serum alkaline phosphatase reaches normal range.

The alkaline phosphatase released by the osteoblasts caused elevation of inorganic phosphorus levels in the serum (Komnenou *et al.*, 2005) [12]. Hence the values of inorganic phosphorus varied in correlation with those of alkaline phosphatase in the affected dogs. The values of serum phosphorous increased initially up to 30th postoperative day in group –I & II and up to the 15th postoperative day in group-III & IV and later declined to normal levels in the present study. The higher levels of inorganic phosphorus in group-II & IV suggested augmented healing process at the fracture site. Suryawanshi *et al.* (2001) [18] and Patil *et al.* (2017) [16] observed increase in the values immediately after fracture fixation to the end of observation period. Whereas; Hegde *et al.* (2007) [9] reported that, the values had increased up to the 60th postoperative day and then declined. The increase in extracellular concentrations of phosphorus results in the deposition of calcium at the fracture site, thus causing ossification of the callus (Komnenou *et al*., 2005) [12].

The Serum calcium values decreased from preoperative and immediate postoperative period to the 30th post-operative period in all the dogs and then increased to normal by the 90th postoperative period. Suryawanshi *et al.* (2001) [18], Hegde *et al.* (2007) [9], Patil *et al.* (2017) [16] and Chaurasia *et al.* (2019) [7] observed a decrease in the values of serum calcium initially, that increased to normal range by the end of observation period. Chaurasia *et al.* (2019) [7] opined that, deposition of excessive calcium at the fracture site might cause a decrease in the levels of serum calcium during earlier phase of postoperative period and termination of the healing process at the fracture site in later phases might cause elevation of the levels.

Table 1: The mean + SE values of alkaline phosphatase in different groups during various periodic intervals.

Group	Pre-operative day	Immediate- P.O.D	7th P.O.D	15th P.O.D	30th P.O.D	60th P.O.D	90th P.O.D	Total Mean ± SE
I	138.00±4.24^{A}	136.50±3.43^{A}	151.80±4.67$^{AB\ a}$	164.22±5.05$^{B\ a}$	165.60±5.09$^{B\ a}$	164.22±5.05^{B}	133.77±3.36$^{A\ b}$	150.58±2.59
II	147.50±9.87	145.00±8.25	163.89±10.96^{ab}	177.00±11.85^{ab}	178.47±11.95^{ab}	177.00±11.85	133.40±7.59^{b}	160.32±4.52
III	152.33±7.96^{B}	151.83±5.23^{B}	174.09±9.10$^{BC\ ab}$	198.03±10.35$^{C\ ab}$	188.89±9.87$^{C\ ab}$	167.57±8.76^{BC}	91.10±3.14$^{A\ a}$	160.55±5.82
IV	154.50±10.88^{B}	148.33±7.15^{B}	196.25±12.93$^{BC\ b}$	219.39±15.45$^{C\ b}$	217.60±15.42$^{C\ b}$	163.77±11.53^{B}	89.00±4.29$^{A\ a}$	169.83±7.81

Means bearing different superscripts within a row (A,B.) and within a column (a, b.) differ significantly (p<0.05).

*Normal physiological range of alkaline phosphatase in dog is 1-114 units/L.

Table 2: The mean + SE values of Serum phosphorus in different groups during various periodic intervals

Group	Pre-operative day	Immediate- P.O.D	7th P.O.D	15th P.O.D	30th P.O.D	60th P.O.D	90th P.O.D	Total Mean ± SE
I	4.31±0.13^{A}	4.27±0.10^{A}	4.74±0.14$^{AB\ a}$	5.13±0.16$^{B\ a}$	5.17±0.16$^{B\ a}$	5.13±0.16^{B}	4.18±0.10^{A}	4.70±0.08
II	4.61±0.31	4.53±0.25	5.12±0.34^{ab}	5.53±0.37^{ab}	5.57±0.37^{ab}	5.53±0.37	4.28±0.23	5.02±0.14
III	4.75±0.24^{AB}	4.74±0.16^{AB}	5.43±0.28$^{BC\ ab}$	6.19±0.32$^{C\ ab}$	5.90±0.30$^{BC\ ab}$	5.23±0.27^{BC}	3.55±0.97^{A}	5.12±0.16
IV	4.82±0.34^{AB}	4.63±0.22^{AB}	6.13±0.40$^{BCD\ b}$	6.85±0.48$^{D\ b}$	6.80±0.48$^{CD\ b}$	5.11±0.36^{ABC}	3.65±0.13^{A}	5.43±0.22

Means bearing different superscripts within a row (A,B.) and within a column (a,b.) differ significantly (p<0.05).
*Normal physiological range of serum phosphorus in dog is 2.9-5.3 mg/ dL.

Table3: The mean + SE values of Serum calcium in different groups during various periodic intervals

Group	Pre-operative day	Immediate- P.O.D	7th P.O.D	15th P.O.D	30th P.O.D	60th P.O.D	90th P.O.D	Total Mean ± SE
I	10.65±0.83	10.78±0.90	10.12±0.79	8.98±0.78^{b}	7.99±0.62	9.58±0.75	10.24±0.86	9.76±0.31
II	10.01±0.85	10.53±0.85	9.21±0.78	8.53±0.68^{ab}	7.51±0.64	9.21±0.78	10.00±0.80	9.29±0.31
III	10.25±0.67^{C}	10.60±0.67^{C}	9.22±0.60^{ABC}	7.42±0.47$^{AB\ ab}$	7.07±0.46^{A}	9.84±0.64^{BC}	10.07±0.64^{BC}	9.21±0.29
IV	10.58±0.87^{C}	10.38±0.84^{C}	8.99±0.74^{ABC}	6.23±0.51$^{A\ a}$	6.56±0.54^{AB}	10.16±0.83^{C}	9.86±0.80^{BC}	8.96±0.37

Means bearing different superscripts within a row (A,B.) and within a column (a,b.) differ significantly (p<0.05).
*Normal physiological range of serum phosphorus in dog is 9.1-11.7 mg/ dL.

Conclusion

Serum alkaline phosphatase and serum phosphorus levels increased during the early stages of fracture healing and gradually declined to normal levels by the late stages of the study in all the groups, whereas serum calcium levels decreased initially and then rose to normal levels by the late stages of the study. This change in value trend effectively reflected the fracture healing state in the current study in distinct groups. Based on the findings of biochemical parameters, group-III and IV animals where biomaterials are employed to bridge the fracture gap had a higher healing rate than the other two groups.

References

1. Allen MJ, Hoffmann WE, Richardson DC, Breur GJ. Serum markers of bone metabolism in dogs. American journal of veterinary research.1998;59:250-254.
2. Aron DN. Stages of bone healing. In: BojrabMJ, Ellison GW, Slocum B, eds. Current Techniques in Small Animal Surgery. 4th ed. Baltimore, PA: Williams and Wilkins; c1998. p. 872-873.
3. Betts CW. Malunion, delayed union, and nonunion. In: Olmstead ML, ed. Small Animal Orthopedics. St Louis, MO: Mosby-Year Book Inc; c1995. p. 277-291.
4. Bhalerao DV. Comparative studies on plate-rod construct and bone plating for long bone fractures in canines. M.V.Sc. Thesis, submitted to the Maharashtra Animal and Fisheries Sciences University, Nagpur, Maharashtra; c2010.
5. Bolger JT. Heterotrophic bone formation and alkaline phosphatase. Archives of Physical Medicine and Rehabilitation. 1975;56:36-39.
6. Chaudhari MM, Ganesh TN, David WPA. Biochemical and pathological studies during compound tibial fracture management using Illizarov's circular external skeletal fixator in dogs. Indian Journal of veterinary surgery. 2000;77:313-316.
7. Chaurasia A, Jawre S, Singh R, Shahi A, Pathak R, Das B, *et al*. Evaluation of haemato-biochemical parameters using different biomaterials in fracture healing of dogs. International journal of current microbial and applied sciences. 2019;8(05):2265-2271.
8. DeCamp CE. Delayed/nonunion fractures. Proceedings of the North American Veterinary Conference. Orlando, FL; c2003. p. 755-756.
9. Hegde Y, Dilipkumar D, Usturge S. Comparative evaluation of biochemical parameters during fracture healing in dogs. Karnataka Journal of Agricultural Sciences. 2007;20(3):694-695.
10. Johnson KA, Watson ADJ. Skeletal diseases. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat. 5thed, Philadelphia, PA: WB Saunders; c2000. p. 1887-1916.
11. Kaderly RE. Delayed union, nonunion and Malunion. In: Slatter D, ed. Textbook of Small Animal Surgery. Philadelphia, PA: WB Saunders; c1993. p. 1676-1685.
12. Komnenou A, Karayannopoulou M, Polizopoulou ZS, Constantinidis TC, Dessiris A. Correlation of serum alkaline phosphatase activity with the healing process of long bone fractures in dogs. Veterinary Clinical Pathology. 2005;34(1):35-38.
13. Kulkarni SD. Studies on treatment of fracture of femur in canines with horn pegs and Kuntscher's nail. M.V.Sc. Thesis submitted to the Marathwada Agricultural University, Parbhani; c2000.
14. Leung KS, Fung KP, Sher AH, Li CK, Lee KM. Plasma bone-specific alkaline phosphatase as an indicator of Osteoblastic activity. Journal of Bone and Joint Surgery. 1993;75B:288-292.
15. Nyman MT, Pavolainen P, Forsius S, *et al*. Clinical evaluation of fracture healing by serum Osteocalcin and alkaline phosphatase. Ann Chir Gynaecology. 1991;80:289-293.
16. Patil M, Dilipkumar D, Shivaprakash BV, Kasaralikar VR, Tikare VP, Ramesh BK. Physiological and haemato-biochemical changes during repair of femur fracture in dogs. The Pharma Innovation Journal. 2017;6(8):381-385.
17. Sousa C, Abreu H, Viegas C, Azevedo J, Reis R, Gomes M, *et al*. Serum total and bone alkaline phosphatase and tartrate-resistant acid phosphatase activities for the assessment of bone fracture healing in dogs. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2011;63(4):1007-1011.
18. Suryawanshi SB, Maiti, Singh GR. A comparative study of two different fixation techniques in management of tibial metaphyseal fracture in dogs. Applied Animal Research. 2001;20:89-98.