www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(3): 577-580

www.thepharmajournal.com
Received: 24-01-2018
Accepted: 25-02-2018

K Manoj Kumar
Post Graduate Student,
Dept. of Veterinary Surgery & Radiology, College of Veterinary Science, Tirupati,
Andhra Pradesh, India

V Devi Prasad
Associate Professor, Dept. of Veterinary Surgery & Radiology, NTR College of Veterinary Science, Gannavaram,
Andhra Pradesh, India

N Dhana Lakshmi
Professor, Dept. of Veterinary Surgery & Radiology, College of Veterinary Science, Tirupati,
Andhra Pradesh, India

NKB Raju
Associate Professor, Dept. of Veterinary Surgery & Radiology, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Correspondence
K Manoj Kumar
Post Graduate Student,
Dept. of Veterinary Surgery & Radiology, College of Veterinary Science, Tirupati,
Andhra Pradesh, India

Evaluation of biochemical parameters for assessment of fracture healing in dogs

K Manoj Kumar, V Devi Prasad, N Dhana Lakshmi and NKB Raju

Abstract
Biochemical parameters like serum calcium, inorganic phosphorus, C-reactive protein and alkaline phosphatase were evaluated during fracture healing in 6 dogs with distal femoral diaphyseal fractures, which were stabilized with String of pearls (SOP) locking plates. Statistical analysis revealed a highly significant difference (P<0.01) in serum calcium, serum alkaline phosphatase and C-reactive protein values in different stages of postoperative intervals. There was no significant variation in serum phosphorus level post-operatively and the values were within the normal range

Keywords: Serum calcium, inorganic phosphorus, C-reactive protein, alkaline phosphatase, fracture, dogs

Introduction
Clinical examination and radiological assessment are the cornerstone of fracture union. Other approaches for the clinical evaluation of bone status include study of bone mineral density (BMD), Radionucleotide scan, bone histomorphometry and biochemical markers. While X-ray, BMD and radionucleotide scan provide information primarily about the bone macrostructure, integrity, quantity and ultimate outcome of healing, only biochemical markers provide a dynamic picture about the underlying process of bone resorption including its turnover, pathogenesis and can differentiate between normal and delayed healing. Furthermore they can be used to monitor short term effects of therapy and provide early indication of any impairment of healing process. Assay procedures of the biochemical markers are inexpensive and can be repeated several times in a single patient. Biochemical markers of bone turnover are generally divided into two subclasses: bone resorption and bone formation markers. The bone resorption markers are related to osteoclast resorption of matrix and include tartarate resistant acid phosphatase and degradation products of type I collagen in protein matrix especially hydroxyproline, telopeptides etc. Bone formation markers are osteocalcin and bone specific alkaline phosphatase produced from osteoblasts (Delmas, 1995) [2]. C-reactive protein is an acute phase reactant, a protein made by the liver and released into the blood within a few hours after tissue injury, the start of an infection, or other cause of inflammation (Scherer *et al*., 2001) [9]. The present study was carried out to assess the degree of bone formation and pain postoperatively using serum calcium, inorganic phosphorus, alkaline phosphatase and C-reative protein estimation respectively after stabilizing the distal femoral diaphyseal fractures with String of pearls (SOP) locking plates in dogs

Materials and methods
Six owner owned dogs of distal femoral diaphyseal fractures presented to the Department of Veterinary Surgery and Radiology, College of Veterinary Science, Tirupati were selected to study the fracture stabilization technique and were fixed were stabilized with String of Pearls (SOP) locking plate along with standard cortical and cancellous screws following standard AO/ASIF principles. Blood samples were collected and serum was separated in all the cases before and after surgery on day 0 and on 7th, 14th, 28th, 45th and 60th day in all the cases and estimated serum calcium, inorganic phosphorus, serum alkaline phosphatase and C-reactive protein. The levels of calcium (mg/dl) in serum were determined by OCPC method (Ortho-cresolphthalein complexone) with kit from Span Diagnostics Ltd (Lin *et al.*, 1999) [6]. The levels of inorganic phosphorus (mg/dl) in serum were determined by Ammonium Molybdate Method with kit from Span Diagnostics Ltd (Young, 2000) [16]. Serum alkaline phosphatase (IU) was estimated by IFCC kinetic assay method (Young, 1997) [15].

C-reactive protein values (mg/dl) were estimated by following latex slide and tube test with kit from Span Diagnostics Ltd (Young, 1997) [15]. The data regarding serum biochemical parameter values were subjected to standard statistical analysis using one way ANOVA as described by Snedecor and Cochran, 1994 using SPSSR 15 software package.

Results and discussion

Serum calcium

The changes in mean ± SE values of serum calcium at different time interval (0 day, 7th, 14th, 28th, 45th and 60th day) are presented in Table 1. Serum calcium levels at all the stages (0 day, 7th, 14th, 28th, 45th and 60th day) differed significantly (P<0.01) with highest values recorded i.e. 11.03 ± 0.21 on 14th day of postoperative period. The pre and postoperative serum calcium mean values showed a significant rise up to 14 days followed by gradual decrease and reaching normally on 60th day postoperatively (Fig 1).

Serum Phosphorus

The mean ± SE values of serum inorganic phosphorus are given in Table 1. There was no significant variation in serum phosphorus level post-operatively and the values were within the normal range (Fig 2).

Serum alkaline phosphatase

The mean ± SE values of serum alkaline phosphatase are given in Table 1. Serum alkaline phosphatase at all the stages (0 day, 7th, 14th, 28th, 45th and 60th day) differed significantly (P<0.01) in different postoperative days. Highest value 115.52 ± 1.89 was observed at 14th day postoperative where as the lowest value 58.85 ± 2.06 on 60th day (Fig 3).

C-reactive protein (CRP)

The mean ± SE values of serum alkaline phosphatase are given in Table 1. CRP values were differed significantly (P<0.01) at various stages with highest values recorded i.e. 39.37 ± 1.77 and 24.09 ± 1.79 on 7th and 14th day of postoperatively (Fig 4).

Biochemical parameters like serum calcium, inorganic phosphorus and alkaline phosphatase enzyme activity during fracture healing were analyzed and evaluated by numerous workers (Hegade, 2007) [4]. The serum calcium mean values showed a significant rise up to 14 days followed by gradual decrease in the value and reaching normally at 60 days of postoperative interval period. The serum calcium level in all the animals fluctuated within normal physiological range. This could be due to severe trauma with comminuted and unstable fractures. The present observations were in accordance with those of Uma Rani and Ganesh, 2003 [13]. The initial decrease in serum calcium was probably due to its increased urinary excretion after traumatic bone injury as stated by Kumar *et al.* (1999) [5], the serum phosphorous mean values showed no significant variation postoperatively and the values were within the normal range. The present observations were in accordance with those of Singh *et al.*, (1976) [11], Contradictory to the present study, Siemens (1970) [10] reported increased phosphorous levels on 30th postoperative day and Nagaraj *et al.* (2003) [8] noticed significant increase in phosphorous levels up to 15th postoperative day.

The serum alkaline phosphatase values significantly increased from preoperative day to 14th day and there after the levels decreased reaching normal by 60th day. Increase in serum alkaline phosphatase level may be due to increased chondroblastic proliferation to cause bone formation during fractured bone repair and also maximum contribution was from the periosteum of destructed bone which was a rich source of serum alkaline phosphatase. The findings were in concurrence with those of Guyton, 1981 and Maiti *et al.* (1999) [7] and Uma Rani and Ganesh (2003) [13]. Contrary to this, Singh *et al.* (1976) [11] reported increased serum alkaline phosphatase activity throughout the study period which was attributed to the muscle, skin trauma and early stage of bone repair. CRP values differed significantly in different stages of postoperative intervals. These elevated values might be because of initial pain due to fractures as opined by Caspi (1984) [1] and surgical trauma according to Yamamoto *et al.* (1993) [14].

Table 1: Mean ± SE Serum biochemical values in pre and postoperative days

Days	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase (IU)	C-reactive protein (µg/ml)
0th	10.22^{a} ± 0.05	5.42^{a} ± 0.36	75.54^{a} ± 0.91	14.74^{a} ± 0.94
7th	10.66^{b} ± 0.05	5.40^{a} ± 0.18	96.30^{b} ± 0.86	39.37^{b} ± 1.77
14th	11.03^{b} ± 0.21	5.37^{a} ± 0.23	115.52^{c} ± 1.89	24.09^{c} ± 1.79
28th	10.19^{a} ± 0.06	5.39^{a} ± 0.29	94.18^{b} ± 0.86	17.09^{a} ± 0.58
45th	10.16^{a} ± 0.03	5.46^{a} ± 0.21	67.91^{d} ± 0.82	14.81^{a} ± 0.64
60th	10.09^{a} ± 0.02	5.29^{a} ± 0.14	58.85^{e} ± 2.06	9.83^{d} ± 0.31

Means with different superscripts (a, b, c, d, e) In six rows of each biochemical parameters differ significantly (P<0.01)

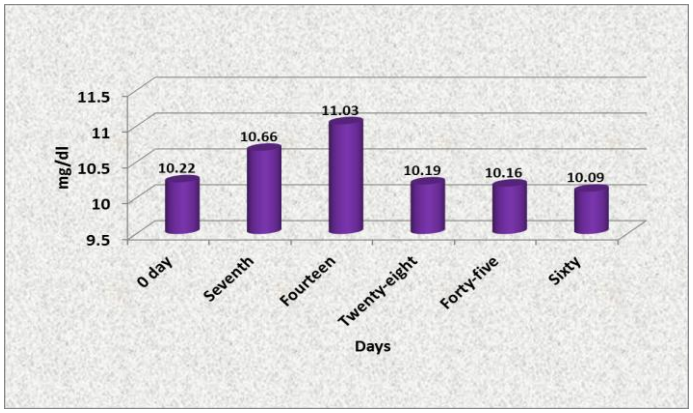

Fig 1: Changes in serum Calcium levels - 1st to 60th Day

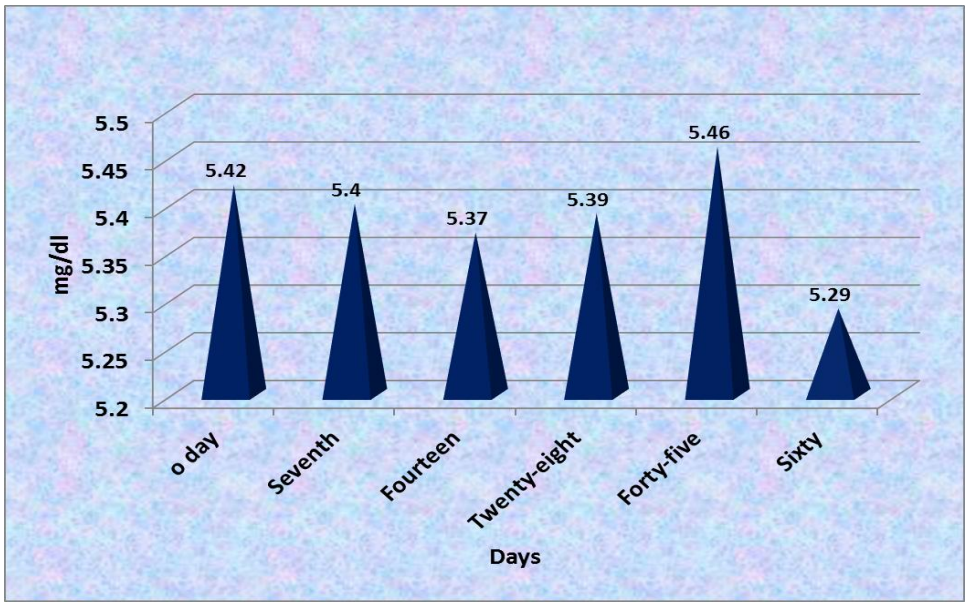

Fig 2: Changes in serum Phosphorus levels - 1st to 60th Day

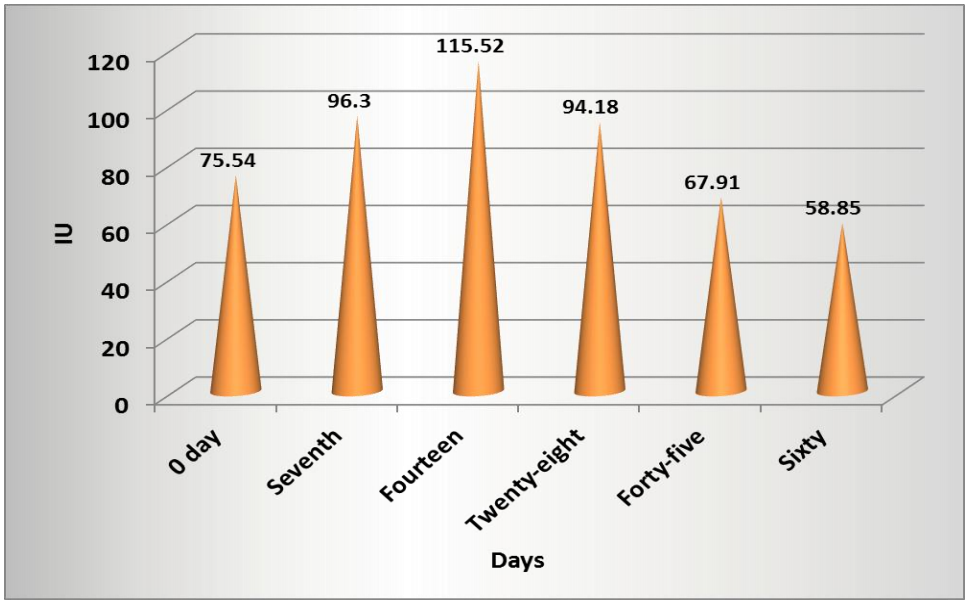

Fig 3: Changes in serum alkaline phosphatase levels - 1st to 60th Day

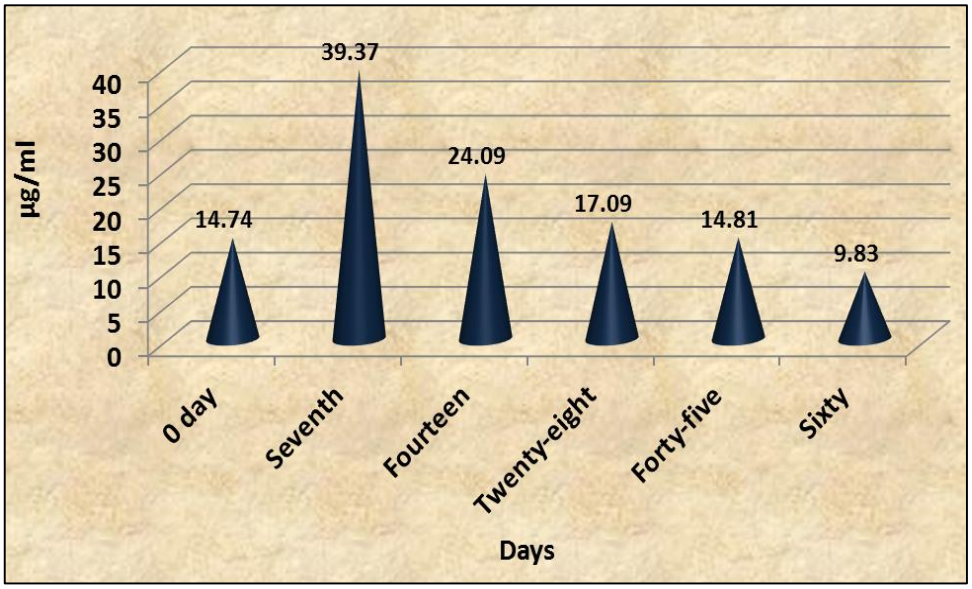

Fig 4: Changes in serum C-reactive protein values - 1st to 60th Day

Conclusion

It was concluded that serum calcium, inorganic phosphorus, alkaline phosphatase and C-reactive protein can be used as useful biochemical markers in assessing the bone formation and pain respectively. These biochemical parameters along with clinical and radiographical examination provide sound knowledge on the degree of bone healing.

References

1. Caspi D, Baltz ML, Snel F, Gruys E, Niv D, Batt RM, Munn EA, Buttvess N, Pepys MB. Isolation and characterization of CRP from the dog. Immunolology. 1984; 53(2):307-313.
2. Delmas PD. Biochemical markers of bone turnover. Acta Orthopaedica Scandinavica 1995; 66:176-82.
3. Guyton AC. Text book of medical physiology. Edn.6, Vol.1, W.B Saunders Co Philadelphia, 1981, 259.
4. Hegade Y, Dilipkumar D, Ustarge S. Comparative evaluation of biochemical parameters during fracture healing in dogs. Karnataka Journal of Agriculture and Science 2007; 20:694-695.
5. Kumar V, Varshney AC, Singh M, Sharma SK, Nigam JM. Haemato -biochemical changes during fracture repair with hydroxyl apatitefibrillarcollagen implants in calves. Indian Journal of Veterinary Surgery. 1999; 20(2):92-93.
6. Lin J, Idee JM, Port M, Diai A, Berthommier C, Robert M *et al*. Interference of magnetic resonance imaging contrast agents with the serum calcium measurement technique using colorimetric reagents. Journal of Pharmaceutical and Biomedical Analysis. 1999; 21(5):931-43.
7. Maiti BK, Sen TB, Sanki S. Haemato-biochemical changes following application of Ilizarov technique in treatment of femur fractures in dogs. Indian Journal of Animal Health. 1999; 38:133-134.
8. Nagaraja BN, Srinivas CL, Jayadevappa SM, Ranganath BN, Vijayasarathy SK. Biochemical and histopathological changes in dogs with femoral fractures immobilized with plastic rods. Indian Journal of Veterinary Surgery. 2003; 24 (2):111-112.
9. Scherer MA, Neumaier M, Von Gumppenberg S. C-reactive protein in patients who had operative fracture treatment. Clinical Orthopaedics and Related Research 2001; 393:287-293.
10. Siemens MG. Calcium inorganic phosphorus and magnesium metabolism in health and in diseases in clinical biochemistry of domestic animals Edn.2, C.E. Academic press, New York. 1970; 1:922-923.
11. Singh H, Lovell JE, Schiller AG, Kenner GH. Serum calcium, phosphorus, alkaline phosphatase levels in dogs during repair of experimental ulnar defects. Indian Veterinary Journal. 1976; 53:862-865.
12. Snecdor GW, Cochran WG. Statistical methods. Edn.8, Oxford and I B H Publishing co. New Delhi, 1994, 1:59.
13. Uma Rani R, Ganesh TN. Study of serum carcium, phosphorus and alkaline phosphatase during fracture healing of femur in goats. Indian Veterinary Journal. 2003; 80:377-378.
14. Yamamoto S, Shida T, Miyaji S, Santsuka H, Fujise H, Mukana K *et al*. Changes in serum CRP levels in dogs with various disorders and surgical trauma. Veterinary Research Communications. 1993; 17(2):85-93.
15. Young D. In effect of preanalytical variables on clinical laboratory tests. Edn.2, AACC Press Washington, D.C, 1977.
16. Young DS. Effects of Drugs on Clinical Laboratory Tests. Edn.5, AACC Press, Washington, D.C, 2000.