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Biochemical changes during repair of long bone fracture repair using string-of-pearls locking plate in dogs

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Abstract
The present study was conducted to evaluate biochemical parameters like serum calcium, phosphorus and alkaline phosphatase during long bones fracture healing in 18 dogs, which were stabilized with String-of-Pearls (SOP) plate. Statistical analysis revealed significant rise ($p<0.01$) up to 30th post-operative day, followed by decrease ($p<0.01$) by 60th post-operative day in these parameters and values were within normal physiological limits throughout the study.

Keywords: Fractures, SOP plate, biochemical parameters

Introduction
Fracture is a clinical condition in which there is partial or complete break in the continuity of the hard tissues like cartilage and bone. Fractures of long bones are commonly encountered in small animal orthopaedics (Harasen, 2003, Kallianpur *et al*., 2018 and Prabhukumar *et al*., 2019) [21, 8, 17]. Bone healing is a complex physiological process and involves numerous mechanisms at tissue and cellular levels. This phenomenon differs from that of soft tissue because of its morphology and composition. Hence, monitoring of fracture healing is necessary to track the rate and pattern of fracture healing for early detection of the complications in healing so that necessary intervention can be done. Mukhopadhyay *et al*. (2011) [11] reported that among various monitoring protocols, serum biochemical analysis provides the early sign of fracture healing, fracture complications and fracture diseases. The present study was carried out with the objective of assessing the fracture healing based on serum calcium, phosphorus and alkaline phosphatase levels after stabilizing the fracture of long bones using String-of-Pearls (SOP) plates in dogs.

Materials and Methods
The study was conducted on 18 clinical cases of long bone fractures in dogs treated by ORIF using locking String-of-Pearls plate used as an internal fixator. Whole venous blood samples were collected and serum was separated in all the cases immediately after surgery and on 15th, 30th and 60th post-operative days. The collected serum was estimated for serum calcium, phosphorus and alkaline phosphatase levels. The level of calcium (mg/dl) in serum was estimated by modified OCPC method using semi auto biochemical analyser (Serum biochemistry analyser-Master T, Hospitex diagnostic, Brentwood, US) with commercially available kit. The level of serum phosphorus (mg/dl) was estimated by phosphomolybdate method using semi auto biochemical analyser with inorganic phosphorus kit. Serum alkaline phosphatase (IU) was estimated by DGKCSCE recommended procedure using semi auto biochemical analyser with alkaline phosphatase kit. The data regarding serum biochemical parameter values were subjected to statistical analysis by using "student t test" (Snedecor and Cochran, 1994) [20].

Results and Discussion
Serum calcium (mg/dL)
The mean ± S.E., values of serum calcium (mg/dL) on post-operative day 0, 15, 30 and 60 were 8.36±0.22, 9.33±0.22, 10.13±0.14 and 9.29±0.21 respectively (Table 1). In dogs the normal serum calcium values ranged between 9.1- 11.7 mg/ dL (Latimer, 2011) [10].

The serum calcium level was significantly increased on 30th post-operative day thereafter it decreases by 60th post-operative day. Increased levels of serum calcium in the initial post-operative period could be attributed to increased osteoclastic activity, leading to resorption of dead bone. Also, mild elevation in serum calcium levels might be due to mineralization process at the fracture site which was in agreement with Bush (1991) [2]. Newton and Nunamaker (1985) [13] opined that acid phosphatase released by the osteoclast first cause demineralization and removal of organic matrix, which may be responsible for increased level of calcium in serum. These findings were in concurrence with Nagaraja *et al*. (2003) [12], Julie (2005) [7], Hegade *et al.* (2007) [5] and Niveditha (2019) [14].

There was significant decrease ($p<0.01$) in the serum calcium levels by 60th post-operative day. This could be attributed to the drainage of the excess calcium from the blood and deposition at the fracture site. The reduction in serum calcium levels represents lowered level of extracellular calcium which stimulates the release of calcium metabolising hormones as opined by Komnenou *et al.* (2005) [9].

Serum phosphorous (mg/dL)

The mean ± S.E., values of serum phosphorous (mg/dL) on post-operative day 0, 15, 30 and 60 were 3.84±0.17, 4.78±0.15, 5.37±0.16 and 4.96±0.12 respectively (Table 1). In dogs the normal phosphorus values ranged between 3.9- 11.7 mg/dL (Latimer, 2011) [10].

The serum phosphorous level was significantly increased on 30th post-operative day thereafter it decreases by 60th post-operative day. The increase in the levels of serum phosphorus indicated the formation of hydroxyappetite during the mineralisation of extra cellular matrix (Komnenou *et al*., 2005) [9]. Similar findings were observed by Paskalev *et al.* (2005) [15], Hegade *et al*. (2007) [5], Rani *et al*. (2012) [18] and Farooq *et al*. (2019) [3].

There was significant gradual decrease ($p<0.01$) in the serum phosphorous levels by 60th post-operative day. Osteoclastic activity leading to resorption of dead bone resulting in gradual decrease in serum phosphorous could be attributed to action as observed in present study.

Serum alkaline phosphatase (U/L)

The mean ± S.E., values of serum alkaline phosphatase (U/L) on post-operative day 0, 15, 30 and 60 were 66.66±1.62, 82.87±2.05, 96.37±2.32 and 80.46±1.03 respectively. In dogs the normal alkaline phosphatase values ranged between 1-114 IU/L (Latimer, 2011) [10].

The serum alkaline phosphatase level was significantly increased on 30th post-operative day thereafter it decreases by 60 day post operatively in all the animals. This was in accordance with the findings of Hegade *et al.* (2007) [5], Phaneendra *et al*. (2018) [16], Chaurasia *et al*. (2019) [1], Niveditha (2019) [14] and Vani *et al*. (2021) [21]. Increase in serum alkaline phosphatase levels might be attributed to increased chondroblastic proliferation to cause bone formation during fractured bone repair and formation of bone matrix (Rani *et al*., 2012 and Singh *et al*., 2017) [18, 19].

The decrease in the levels of alkaline phosphatase might be indicative of cessation of osteoblastic activity and receding of the values towards its base value due to ossification and consolidation of fractured bone. As osteoblast secretes large quantities of alkaline phosphatase, which is involved in the process of matrix formation and its mineralization. Alkaline phosphatase is believed to either increase the concentration of local inorganic phosphate or inorganic pyrophosphate that is necessary for fracture healing.

Table 1: Mean ± SE serum biochemical values on post-operative days

Post-operative days	Serum calcium (mg/dL)	Serum phosphorous (mg/dL)	Serum alkaline phosphatase (U/L)
0th day	8.36±0.22	3.84±0.17	66.66±1.62
15th day	9.33±0.22**	4.78±0.15**	82.87±2.05**
30th day	10.13±0.14**	5.37±0.16**	96.37±2.32**
60th day	9.29±0.21**	4.96±0.12**	80.46±1.03**

Means with superscript** four rows of each biochemical parameters differ significantly ($p<0.01$)

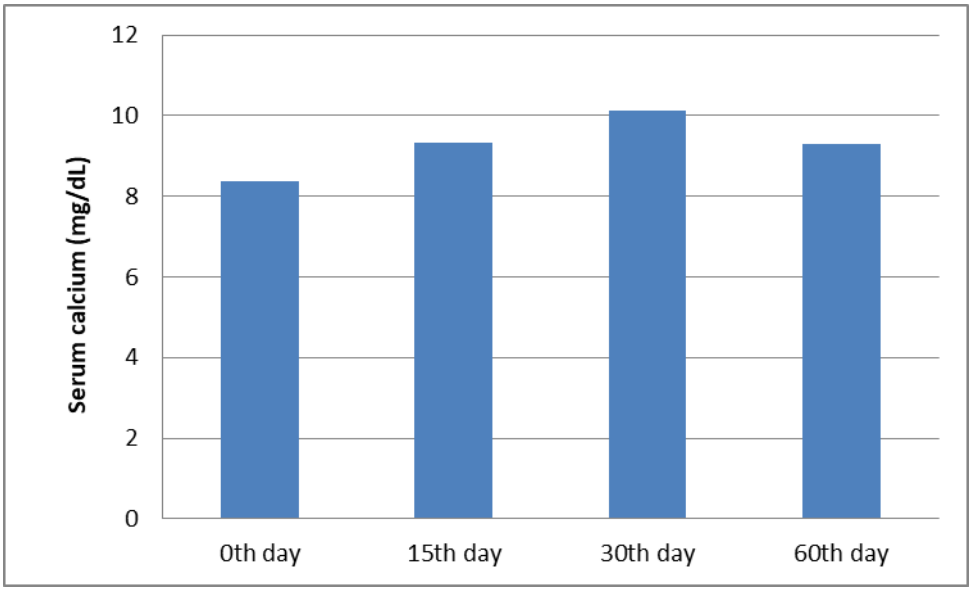

Fig 1: Graphs showing values of serum calcium (mg/dL) on different post-operative days

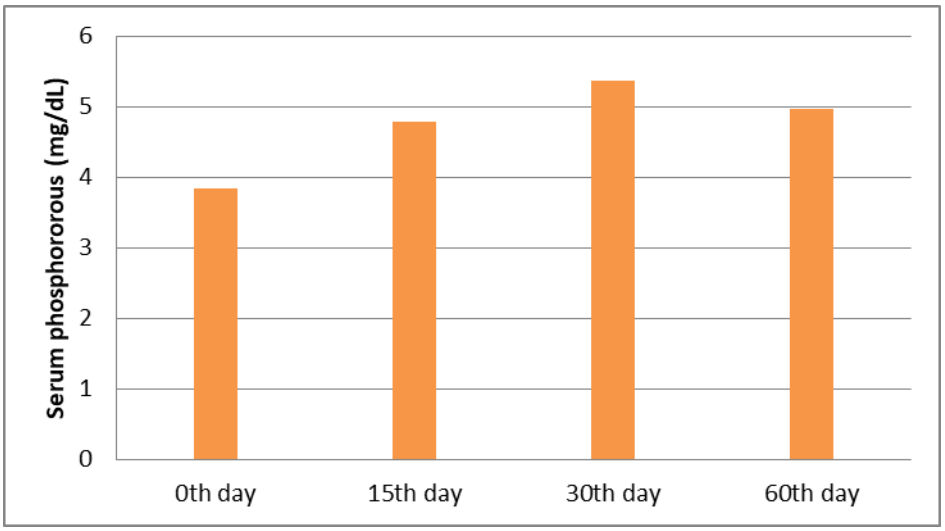

Fig 2: Graphs showing values of serum phosphorous (mg/dL) on different post-operative days

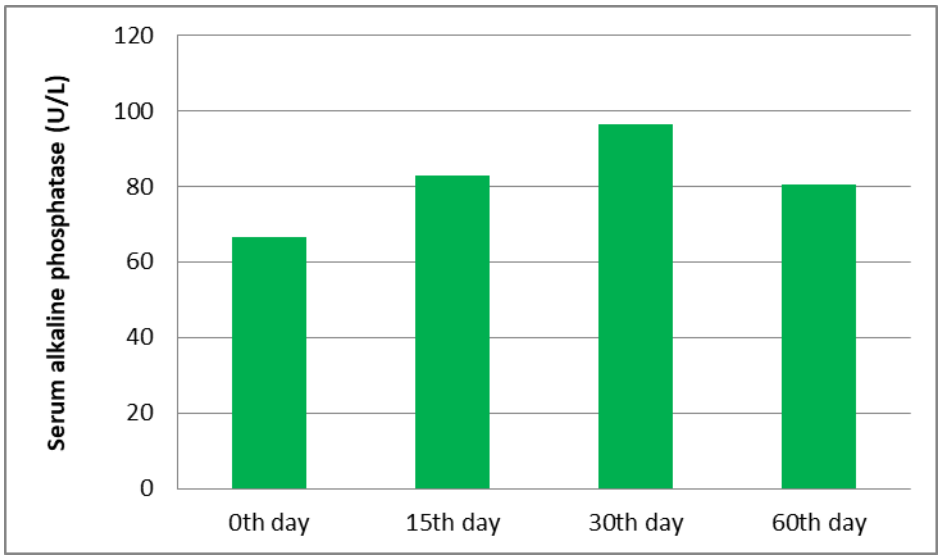

Fig 3: Graphs showing values of serum alkaline phosphatase (U/L) on different post-operative days

Conclusion

The present study confirmed that serum calcium, phosphorus and alkaline phosphatase levels were within the normal range, indicating minimal callus formation during fracture healing. The SOP plate provided stable and rigid fixation of the fracture with rapid healing of the fracture. These biochemical parameters may need to be combined with other parameters for a realistic assessment of the fracture healing process.

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