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Evaluation of biochemical parameters for assessment of long bone fracture healing in young dogs subjected to bone plating

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Abstract
Biochemical parameters like serum calcium, inorganic phosphorus, C-reactive protein and alkaline phosphatase were evaluated during fracture healing in 6 young dogs after stabilizing radius or tibial fractures by Elastic Plate Osteosynthesis technique using 2.0/2.7 mm Veterinary Cuttable Plate. Statistical analysis revealed a highly significant difference (P<0.01) in serum calcium and serum alkaline phosphatase 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, alkaline phosphatase, fracture, young dogs

Introduction
Femur fracture accounts for highest percentage of fracture occurrence in small animals (Simon *et al*., 2010; Kushwaha *et al*., 2011) [19, 10]. Fracture healing is a complex physiological process it involves many types of cells, biochemical regulating factors and expression of several thousand genes (Eihorn, 1998) [4]. Clinical examination and radiographical assessment are the cornerstone for 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 outcome of healing, only biochemical markers provide a dynamic picture about the underlying process of bone remodelling including its turnover, pathogenesis and can differentiate between normal and delayed healing (Mukhopadhyay *et al.* 2011) [14]. The present study was carried out to assess the degree of bone formation postoperatively using serum calcium, inorganic phosphorus and alkaline phosphatase estimation respectively after stabilizing radius or tibial fractures by Elastic Plate Osteosynthesis technique using 2.0/2.7 mm Veterinary Cuttable Plate.

Materials and Methods
Six young dogs aged below one year presented to the Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram with unstable radius or tibia fracture were selected to study the fracture stabilisation by Elastic Plate Osteosynthesis technique using 2.0/2.7 mm Veterinary Cuttable Plate following standard AO/ASIF principles. Blood sample was collected and serum was separated in all the cases on preoperative, immediate, 15th, 30th and 60th postoperative days for estimation of serum calcium, phosphorous and alkaline phosphatase.
The serum calcium levels (mg/dL) were determined by Arsenazo- III method with kit supplied by Asritha Diatech India Pvt. Ltd., Hyderabad, India. The serum inorganic phosphorus (mg/dL) levels were estimated by Molybdate U.V. method using kit marketed by Coral Clinical Systems, a division of tulip diagnostics (P) Ltd., Uttarakhand, India. Serum alkaline phosphatase (IU) was estimated by Tris Carbonate buffer, Kinetic Kit method using Erba alkaline phosphatase kit manufactured by Transasia Bio-meicals Ltd., Baddi, Solan District, Himachal Pradesh, India. The data collected were statistically analyzed using S P S S-ANOVA Post Hoc test in Tukey H S D.

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, 15th, 30th and 60th day) differed significantly (P<0.01) with highest values recorded i.e. 13.63^{2}±0.51 on 15th day of postoperative period. The pre and postoperative serum calcium mean values showed a significant rise up to 15 days followed by gradual decrease and reaching normally on 60th day postoperatively.

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.

Serum alkaline phosphatase

The mean ± SE values of serum alkaline phosphatase are given in Table 1. Elevation in the serum alkaline phosphatase levels was observed up to 15th post-operative day and reached a peak on 15th post-operative day with a gradual return to normal base value on 60th post-operative day with statistically significant difference between day immediate and 15th, immediate and 30th, 15th and 30th, 15th and 60th and 30th and 60th post-operative days whereas no significant difference was observed between immediate and 60th post-operative days (P<0.01).

Biochemical parameters like serum calcium, inorganic phosphorus and alkaline phosphatase enzyme activity during fracture healing were analyzed and evaluated by numerous workers (Hegade, 2007) [6]. The post-operative serum calcium mean values showed a significant rise upto 15 days post-surgery and reached a peak on 15th post-operative day followed by gradual decline and reaching base value on 30th day post-surgery. Significant difference in serum calcium levels was observed (P<0.01) between 15th and 60th postoperative days whereas no significant difference was observed between immediate and 15th, immediate and 30th, immediate and 60th, 15th and 30th and 30th and 60th post-operative days (P<0.01). The increase in serum calcium level could be attributed to mineralization process at the osteotomy site (Nagaraja *et al.*, 2003) [15]. Similar findings were observed by Nagaraja *et al.* (2003) [15] and Kumar *et al.* (2018). On the contrary Uma Rani *et al.* (2012) [21] observed reduction in serum calcium level on 3rd post-operative day followed elevation up to 60th post-operative day. Daniella (2017) [2] found no significant difference in the serum calcium levels upto 60 days post-surgery. Julie (2005) [7] observed significant decrease in serum calcium level by 4th week.

No statistically significant difference was observed between the time periods with regard to serum phosphorus which was in accordance with Daniella (2017) [2] and Kumar *et al.* (2018) [8]. Contradictory to the present study, Siemens (1970) [18] reported increased phosphorous levels on 30th postoperative day and Nagaraja *et al.* (2003) [15] noticed significant increase in phosphorous levels upto 15th postoperative day and attributed the same to necrotic disintegration of cells at the fracture site.

Elevation in the serum alkaline phosphatase levels was observed up to 15th postoperative day and reached a peak on 15th postoperative day with a gradual return to normal base value on 60th postoperative day with statistically significant difference between day immediate and 15th, immediate and 30th, 15th and 30th, 15th and 60th and 30th and 60th post-operative days whereas no significant difference was observed between immediate and 60th post-operative days (P<0.01) which was in accordance with Phaneendra *et al.* (2016) and Kumar *et al.* (2018) [8]. On the contrary, Daniella (2017) [2] noticed a non-significant decrease in serum alkaline phosphatase levels from the initial base value upto 60 days post-surgery. The increase in levels of serum alkaline phosphatase was due to increased chondroblastic proliferation to cause bone formation during fracture repair and also maximum contribution was from the periosteum of destructed bone which was a rich source of serum alkaline phosphatase (Guyton, 1981, Hegade *et al.*, 2007 and Mahendra *et al.*, 2007) [5, 6, 12]. Nagaraja *et al.* (2003) [15] and Patil *et al.* (2017) [16] observed no significant variation in the serum alkaline phosphatase values.

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

Day	Calcium (mg/dL)	Phosphorous (mg/dL)	Alkaline phosphatase (IU)
Immediate	11.43^{12}±0.85	4.99±0.32	74.74^{1}±1.53
15	13.63^{2}±0.51	5.21±0.31	101.00^{3}±0.99
30	10.83^{12}±0.51	4.88±0.24	90.44^{2}±1.40
60	10.59^{1}±0.39	4.85±0.18	71.06^{1}±0.92

Means with different superscripts (numerical) between days in a group differ significantly (P<0.01).

Conclusion

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

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