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GV Ashok Kumar Reddy
M.V.Sc Scholar, Department of
Veterinary Surgery and
Radiology, College of Veterinary
Science, Rajendranagar, PVNR
TVU, Hyderabad, Telangana,
India

V Gireesh Kumar
Professor, Department of
Veterinary Surgery and
Radiology, College of Veterinary
Science, Rajendranagar, PVNR
TVU, Hyderabad, Telangana,
India

KBP Raghavender
Professor and University Head,
Department of Veterinary
Surgery and Radiology, College
of Veterinary Science,
Rajendranagar, PVNR TVU,
Hyderabad, Telangana, India

D Pramod Kumar
Professor and University Head,
Department of Veterinary
Anatomy and Histology, College
of Veterinary Science,
Rajendranagar, PVNR TVU,
Hyderabad, Telangana, India

Corresponding Author:
GV Ashok Kumar Reddy
M.V.Sc Scholar, Department of
Veterinary Surgery and
Radiology, College of Veterinary
Science, Rajendranagar, PVNR
TVU, Hyderabad, Telangana,
India

Evaluation of haemato-biochemical parameters for assessment of fracture healing in dogs

GV Ashok Kumar Reddy, V Gireesh Kumar, KBP Raghavender and D Pramod Kumar

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Abstract

The present study was conducted to evaluate haematological and biochemical parameters were evaluated during fracture healing in 7 dogs with femoral fractures stabilized with string of pearls locking plates. Statistical analysis revealed a highly significant difference ($P < 0.01$) in haemoglobin, packed cell volume, total erythrocyte and total leucocyte counts, serum calcium, inorganic phosphorous and serum alkaline phosphatase in different post-operative intervals. No significant difference was observed in serum phosphorous level post-operatively and the values were within the normal physiological range.

Keywords: Haemoglobin, packed cell volume (PCV), serum calcium, serum alkaline phosphatase and fracture healing

Introduction

Femur fractures accounts for highest percentage of fracture occurrence in small animal patients (Gahlod *et al.* 2004, Simon *et al.* 2010, and Kallianpur *et al.* 2018) [4, 14, 8]. Fracture healing is a complex physiological process it involves many types of cells, biochemical regulating factors and expression of several thousand genes (Einhorn, 1998) [3]. 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) [12]. These biochemical markers can also be used to monitor short term effects of therapy and provide early indication of any impairment of healing process. The present study was undertaken to evaluate haemato-biochemical parameters in fracture healing, where the femoral fractures were stabilized with string of pearls locking plates in dogs.

Materials and methods

Seven dogs with femoral fractures presented to Department of Veterinary Surgery and Radiology, College of Veterinary Science, Rajendranagar, Hyderabad were selected to the study the fracture stabilization technique and were fixed with String of Pearls (SOP) locking plate system (SOP) using standard AO/ASIF principles. Blood samples were collected and serum was separated in all the cases preoperatively and on 15th and 45th days post-operatively. Haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count, serum calcium, serum alkaline phosphatase and phosphorous were the parameters evaluated. Haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count were estimated on the above said days as per the methods described by Jain (1993) [6]. The levels of calcium (mg/dL) in serum were determined by OCPC method (ortho-cresolphthalein complexone) (Lin *et al.* 1999) [9]. The levels of inorganic phosphorus (mg/dL) in serum were determined by Phosphomolybdate Method (Young, 1997) [20]. Serum alkaline phosphatase (U/L) was estimated by International Federation of Clinical Chemistry (IFCC) kinetic assay method (Young, 1993) [19]. The data regarding haematological and serum biochemical values were subjected to standard statistical analysis using one way ANOVA as described by Snedecor and Cochran (1994) [17] using Statistical Package for the Social Sciences (SPSS) 15 software package.

Results and Discussion

The details of the haematological and serum biochemical parameters were as follows and are given in tables 1 and 2 respectively.

Table 1: Haematological parameters observed in the present study (Mean \pm SE)

Parameter	Pre-operative	Post-operative	
	Day before surgery	15 th day	45 th day
Hb (g/dL)	8.98 ^a \pm 0.22	10.27 ^b \pm 0.33	11.14 ^c \pm 0.24
PCV (%)	30.81 ^a \pm 0.85	37.44 ^b \pm 0.61	43.91 ^c \pm 0.23
TEC ($\times 10^6/\mu\text{L}$)	5.01 ^a \pm 0.17	6.12 ^b \pm 0.09	7.09 ^c \pm 0.11
TLC ($\times 10^3/\mu\text{L}$)	12.29 ^a \pm 0.19	11.65 ^b \pm 0.13	10.45 ^c \pm 0.14

Means bearing different superscripts (a, b and c) in the same row differ significantly ($P < 0.01$).

Table 2: Serum Biochemical parameters observed in the present study (Mean \pm SE)

Parameter	Pre-operative	Post-operative	
	Day before surgery	15 th day	45 th day
Alkaline Phosphatase (U/L)	94.81 ^a \pm 0.47	114.90 ^b \pm 0.33	81.5 ^c \pm 0.73
Calcium (mg/dL)	8.90 ^a \pm 0.14	11.63 ^b \pm 0.30	10.35 ^c \pm 0.25
Phosphorous (mg/dL)	5.02 \pm 0.09	5.18 \pm 0.08	5.25 \pm 0.11

Means bearing different superscripts (a, b and c) in the same row differ significantly ($P < 0.01$).

Haemoglobin (g/dL)

The mean \pm SE values of haemoglobin on day before surgery and on 15th and 45th day of surgery were found to be 8.98 \pm 0.22, 10.27 \pm 0.33 and 11.14 \pm 0.24 g/dL respectively. Haemoglobin values differed significantly ($P < 0.01$) among the three intervals. Highest value 11.14 \pm 0.24 g/dL was observed at 45th post-operative day, whereas the lowest value 8.98 \pm 0.22 g/dL was observed on day before surgery.

Packed cell volume (%)

The mean \pm SE values of packed cell volume on day before surgery and on 15th and 45th day of surgery were found to be 30.81 \pm 0.85, 37.44 \pm 0.61 and 43.91 \pm 0.23% respectively. Significant difference ($P < 0.01$) was observed among the packed cell volumes between three intervals. Highest value of 43.91 \pm 0.23% was observed at 45th post-operative day, whereas the lowest value 30.81 \pm 0.85% was observed on day before surgery.

Total Erythrocyte Count ($\times 10^6/\mu\text{L}$)

The mean \pm SE values of total erythrocyte count on day before surgery and on 15th and 45th day of surgery were found to be 5.01 \pm 0.17, 6.12 \pm 0.09 and 7.09 \pm 0.11 $\times 10^6/\mu\text{L}$ respectively. Total erythrocyte count at the three intervals differed significantly ($P < 0.01$). Highest value of 7.09 \pm 0.11 $\times 10^6/\mu\text{L}$ was observed at 45th post-operative day, whereas the lowest value 5.01 \pm 0.17 $\times 10^6/\mu\text{L}$ was observed on day before surgery.

Total Leukocyte Count ($\times 10^3/\mu\text{L}$)

The mean \pm SE values of total leukocyte count on day before surgery and on 15th and 45th day of surgery were found to be 12.29 \pm 0.19, 11.65 \pm 0.13 and 10.45 \pm 0.14 $\times 10^3/\mu\text{L}$ respectively. Total leukocyte count at the three intervals differed significantly ($P < 0.01$). Highest value of 12.29 \pm 0.19 $\times 10^3/\mu\text{L}$ was observed on day before surgery, whereas the lowest value 10.45 \pm 0.14 $\times 10^3/\mu\text{L}$ was observed on 45th post-operative day.

Serum Calcium (mg/dL)

The mean \pm SE values of serum calcium on day before surgery and on 15th and 45th day of surgery were found to be 8.90 \pm 0.14, 11.63 \pm 0.30 and 10.35 \pm 0.25 mg/dL respectively. Serum calcium levels differed significantly ($P < 0.01$) with the highest value 11.63 \pm 0.30 mg/dL was observed on 15th post-operative day.

Serum Alkaline Phosphatase (U/L)

The mean \pm SE values of serum alkaline phosphatase on day before surgery and on 15th and 45th day of surgery were found to be 94.81 \pm 0.47, 114.90 \pm 0.33 and 81.50 \pm 0.73 U/L respectively. Standard statistical analysis by one way ANOVA method revealed that there was significantly different ($P < 0.01$) among the three intervals. Highest value 114.90 \pm 0.33 U/L was observed on 15th post-operative day, whereas, the lowest value 81.50 \pm 0.73 U/L was observed on 45th post-operative day.

Serum Phosphorous (mg/dL)

The mean \pm SE values of serum phosphorous on day before surgery and on 15th and 45th day of surgery were found to be 5.02 \pm 0.09, 5.18 \pm 0.08 and 5.25 \pm 0.11 mg/dL respectively. Statistical analysis revealed that there was no significant difference in serum phosphorous levels in three intervals. The serum phosphorous levels were within the normal range.

The Haemoglobin level and Packed cell volume on the 15th and 45th post-operative days were statistically higher when compared to day before surgery. The total erythrocyte count was significantly elevated on 45th post-operative day when compared to day before surgery. This showed that progressive increase of haemoglobin, packed cell volume and total erythrocyte count on post-operative days indicating erythropoiesis. However, all the values were within the normal physiological limits throughout the course of the present study. This finding was in agreement with the findings of Singh *et al.* (2008) [16].

The total leukocyte count was higher on pre-operative day before surgery when compared to post-operative days. Physiological reduction in total leukocyte count was reported to be suggestive of gradual decrease in inflammatory reaction (Maiti *et al.* 1999) [11]. Leucocytosis occurred in conditions where there was corticosteroid release in state of stress, pain, anaesthesia, trauma and surgical manipulation.

The mean serum calcium values showed a significant rise on the 15th day followed by decrease in the value and reaching normal at 45 days of post-operative interval period. The serum calcium level in all the animals fluctuated within normal physiological range. This could be due to severe trauma associated with comminuted and unstable fractures. The present observations were in accordance with those of Bush (1991) [1], Nagaraja *et al.* (2003) [13] and Uma Rani and Ganesh (2003) [18].

The serum alkaline phosphatase values significantly increased from pre-operative day to 15th day indicating increased chondroblastic proliferation to cause bone formation during bone repair (Maiti *et al.* 1999) [11]. Increase in the serum alkaline phosphatase resulted from the periosteum of destructed bone which was a rich source of alkaline phosphatase. The findings were in concurrence with those of Singh *et al.* (1976) [15], Uma Rani and Ganesh (2003) [18], Julie (2005) [7], Hegde *et al.* (2007) [5] and Mahendra *et al.* (2007) [10]. The serum alkaline phosphatase levels reached normal levels by the 45th post-operative day indicating quiescence at

the fracture site.

The serum phosphorous mean values showed no significant variation post-operatively and the values were within the normal range. The present observations were in accordance with those of Singh *et al.* (1976)^[15], Chandy (2000)^[2] and Mahendra *et al.* (2007)^[10].

Conclusion

Based on the results in the present study, it can be concluded that haematological and biochemical parameters can be used as useful markers in assessing the fracture healing. These haematological, biochemical parameters along with clinical and radiographic examination provide excellent detail on the degree of fracture healing.

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