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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 2116-2119
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www.thepharmajournal.com
Received: 25-08-2023
Accepted: 28-09-2023

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Comparative evaluation of haematological and biochemical parameters in femur fracture in dogs

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Abstract
The present study was conducted on 12 clinical cases of dogs having femur fracture presented to the Veterinary clinical complex, Nagpur Veterinary College, Nagpur. Divided into two equal groups, viz. Group I and Group II consisting of six animals each and which was immobilized by locking compression bone plating and string of pearl bone plating. The blood samples was collected on different intervals for the estimation of haematological parameters haemoglobin (g/dL), packed cell volume (%), total erythrocyte count (x 10^6/cu.mm), total leucocyte Count (x10^7/cu.mm) and differential leucocyte count (%) and biochemical parameters like serum calcium (mg/dl), serum phosphorus (mg/dl) and alkaline phosphatase (mg/dl). The haematological values are with in the normal ranges throughout the duration of healing process. The biochemical observation recorded as serum alkaline phosphatase vales are significant in both the groups whereas serum calcium and serum phosphrous vales are with the normal ranges throughout in the duration of healing process.

Keywords: Fracture, haematological parameters, biochemical parameters, dogs

Introduction
Bone is a specialized type of connective tissue that consists cells, fibers and ground substance. Fractures in bones are more commonly occurs due to an acute non-physiological forces that exceeds ultimate tensile strength over bone (Burns, 2010) [2]. Preservation of intramedullary and periosteal vascularization, anatomical reduction, interfragmentory compression of bone pieces and prompt restoration of normal locomotor function are the basic objectives of fracture treatment (Newton, 1985) [8]. The assessment of haematological and biochemical parameters during fracture healing to identify fluctuations, if any in fracture healing after rigid stabilization are important (Phaneendra *et al*., 2016) [10]. The bone resorption and bone formation these are two biochemical markers generally evaluated in fracture healing. The biochemical markers only provide a dynamic picture about the underlying process of bone resorption including its turnover, pathogenesis and can differentiate between normal and delayed healing and they can be used to monitor short term effects of therapy and provide early indication of any impairment of healing process (Kumar *et al.*, 2018) [5]. The haematological parameters like TLC, neutrophil count were assessed to study the presence of any infection in the post-operative period of fracture stabilization (Umeshwori *et al.*, 2015) [14]. The present study was carried out to assess the degree of bone formation, pain elicited and infection rate at fracture site postoperatively using different serum and blood parameters after stabilizing the femur fracture by using different plates to repair femur in dog.

Materials and Methods
The present study was conducted on 12 clinical cases of dogs having femur fracture presented to the Veterinary clinical complex, Nagpur Veterinary College, Nagpur, divided into two equal groups, viz. Group I and Group II consisting of six animals each. In Group I, fracture was immobilized by locking compressive bone plating and In Group II fracture was immobilized by string of pearl bone plating. The blood samples to estimate the haematological parameters was collected on before surgery, after surgery,$7^{th,}$ 14^{th}, 28^{th} and 45^{th} day in EDTA vials from the recurrent tarsal vein. The estimation of haematological parameters was done by semi-automatic haematological analyser (Mindray, BC – 2800 Vet Auto Hematology Analyzer). The different parameters estimated as follows, Haemoglobin (g/dL), Packed cell volume (%), Total erythrocyte count (x 10^6/cu.mm), Total leucocyte Count (x10^7/cu.mm) and Differential leucocyte count (%).

The biochemical parameters, the blood was collected in vacutainers containing clot activator, from a recurrent tarsal vein and the vials was kept in tilted position for one hour and serum was separated and collected in Eppendorf tubes. The estimation of biochemical parameters at before surgery, after surgery, 7th, 14th, 28th and 45th day after surgery for different parameters like Serum calcium (mg/dl), Serum phosphorus (mg/dl) and Alkaline phosphatase (mg/dl).

Results and Discussion

In present study, total 12 clinical cases of the dogs with fracture of femur presented at Veterinary clinical complex, Nagpur Veterinary College Nagpur divided into two groups, each with six animals Group I and Group II. In Group I, locking compression bone plating was done and in Group II, string of pearl plating was done to immobilise the fracture. In fracture cases, the inflammatory phase is said to commence immediately after the fracture and last until cartilage or bone formation begins. After the onset of inflammation, the number of neutrophils increased, as they are the first line of defence.

Hemoglobin (mg/dl): In the present study, the mean haemoglobin concentration ranged from 9.95±0.21 to 10.41±0.15 gm/dl observed in Group I, while in Group II, it was ranged from 9.97±0.16 to 10.41±0.21 gm/dl (Table 1). A range-consistent, non-significant fluctuation in haemoglobin concentration was observed at various time intervals in both the groups. The decrease in haemoglobin is due to the transfer of fluids from the extravascular compartment to the intravascular compartment in order to maintain normal cardiac output or to the accumulation of circulating blood cells in the spleen as a result of decreased sympathetic activity (Singh *et al*., 2015) [13]. Similar findings observed by Dakhane (2021) [3].

Table 1: Mean (±SE) of hemoglobin in two treatment groups

	0 day	1st day	7th day	14th day	28th day	45th day
Group I	9.68±0.18	9.81±0.19	10.3±0.23	10.41±0.15	9.93±0.20	9.95±0.21
Group II	9.70±0.16	9.80±0.16	10.11±0.19	10.41±0.21	10.08±0.18	9.88±0.20

The mean concentration of hemoglobin between groups at different interval revealed non-significant difference (p>0.05).

Packed Cell Volume (%): The average values for packed cell volume percentage in Group I and Group II ranged from 27.45±0.87 % to 27.91±0.83% and 29.61±0.89 to 30.13±1.43 % (Table 2). Similar observation also noted by Patil *et al.*, (2017) [9] and Dakhane *et al.*, (2021) [3].

Table 2: Percentage of Packed cell volume in all groups of dogs at different interval

	Before	After	7th day	14th day	28th day	45th day
Group I	26.85±0.78	26.98±0.78	27.46±0.79	27.91±0.83	27.76±0.86	27.45±0.87
Group II	27.7±0.83	27.8±0.83	28.11±0.88	28.41±0.88	28.28±0.90	28.05±0.89

Mean packed cell volume between groups revealed that there was non-`significant difference at different interval (p>0.05).

Platelets (lac/cumm): The mean platelet count ranged from 2.68±0.18 to 3.41±0.15 lac/cumm, was observed in Group I whereas in Group II, ranged from 3.2±0.36 to 3.91±0.38 lac/cumm (Table 3). The elevated values on day zero may be a result of systemic inflammatory alterations following fracture and then after 14th day it was decreases. Similar observation also noted by Dakhane *et al*., (2021) [3].

Table 3: Platelet count in all dogs of different groups at different interval

	Before	After	7th day	14th day	28th day	45th day
Group I	2.68±0.18	2.81±0.19	3.3±0.23	3.41±0.15	2.93±0.20	2.9±0.22
Group II	3.2±0.36	3.3±0.36	3.61±0.37	3.91±0.38	3.75±0.35	3.55±0.36

The average count of platelets between groups revealed non- significant difference at different interval (p>0.05).

Total leucocyte count (million/cumm): The mean total leucocyte count ranged from 9.81±0.25 to 10.48±0.27 million/cmm in Group I, whereas in Group II, it ranged from 11.71±0.25 to 12.35±0.28 million/cmm (Table 4). The elevated values on day zero may be a result of systemic inflammatory alterations following fracture. Leucocytosis occurs when there is corticosteroid release in states of tension, pain, anaesthesia, trauma and surgical manipulation was observed by Al-Bulushi *et al*. (2017) [1] and Dakhane *et al.* (2021) [3] reported similar findings.

Table 4: Total leucocyte count in two treatment group

	Before	After	7th day	14th day	28th day	45th day
Group I	9.71±0.25	9.81±0.25	10.2±0.29	10.48±0.27	10.35±0.28	10.08±0.30
Group II	11.71±0.25	11.81±0.25	12.26±0.29	12.31±0.35	12.35±0.28	12.08±0.30

Mean Total leucocyte count between groups observed non significant difference (p>0.05).

Differential leucocyte count

1. Neutrophils (%): The mean neutrophil concentrations range from 78.867±0.47 to 81.8±0.82 % in Group I, whereas in Group II, the range was 79.233±0.17 to 80.8±0.55 % (Table 5).

Table 5: Mean value (±SE) of Total Neutrophil at different time intervals

	O day	1st day	7th day	14th day	28th day	45th day
Group I	81.833±0.52	81.2±0.78	80.583±0.74	79.767±1.02	81.8±0.82	78.867±0.47
Group II	80.45±0.72	80.8±0.55	79.233±0.17	80.253±0.47	80.533±0.49	80.433±0.29

Mean neutrophil count between groups observed non significant difference (p>0.05).

2. Eosinophils (%): The mean eosinophil concentrations range from 0.99±0.17 to 1.25±0.12 %, whereas in Group II, the range was 0.81±0.06 to1.102±0.11% (Table 6).

Table 6: Mean value (±SE) of Total Eosinophils at different time intervals

	O day	1st day	7th day	14th day	28th day	45th day
Group I	1.048±0.154	1.108±0.103	1.25±0.12	1.017±0.16	0.99±0.17	1.122±0.11
Group II	1.102±0.11	1.093±0.15	1.027±0.07	0.81±0.06	1.013±0.18	1.058±0.19

Mean eosinophil count between groups observed non significant difference (p>0.05).

3. Lymphocyte (%): In Group I, mean lymphocye concentrations range from 17.383±1.72 to 21.367±1.04%, whereas in Group II, the range was 18.617±0.91to21.333±1.18 % (Table 7).

Table 7: Mean value (±SE) of Total Lymphocyte at different time intervals

	O day	1st day	7th day	14th day	28th day	45th day
Group I	19.567±1.75	17.383±1.72	18.467±1.12	19.667±1.03	20.983±0.35	21.367±1.04
Group II	19.933±0.61	20.117±0.98	21.333±1.18	20.25±1.02	18.617±0.91	19.33±0.43

Mean Lymphocyte count between groups observed non significant difference (p>0.05).

4. Monocyte (%): In Group I, mean monocyte concentrations range from 17.383±0.63 to 20.983±0.73%, whereas in Group II, the range was 18.617±0.48 to 21.333±0.94% Table 8.

Table 8: Mean value (±SE) of Total Monocyte at different time intervals

	O day	1st day	7th day	14th day	28th day	45th day
Group I	19.567±0.74	17.383±0.63	18.467±0.95	19.667±0.94	20.983±0.73	21.367±0.97
Group II	19.933±0.82	20.117±0.73	21.333±0.94	20.25±0.81	18.617±0.48	19.33±0.62

Mean Monocye count between groups observed non significant difference (p>0.05).

The neutrophil, lymphocyte, eosinophil and monocyte count values obtained in this analysis were well within the physiological range during fracture healing and similar finding observed by Mirajkar (2018) [7].

Biochemical Parameter: Blood sample was collected aseptically from the cephalic or saphenous vein prior to the surgery and subsequently on the day 0th, 7th, 14th, 28th and 45th daypost-operatively for estimation of the alkaline phosphatase, serum calcium and serum phosphorus.

1. Serum Alkaline phosphatase

In Group I, mean alkaline phosphatase ranged from 134.31±6.31 to 221.06±8.32 IU/L, whereas in Group II, it was ranged from 222.58±14.11 to 222.58±14.11 IU/L (Table 9). The serum alkaline phosphatase values in animals of group I was 175.26±16.29 IU/L on day 0, however the values were found elevated on day 7th and 14th .e. 204.46±16.25 IU/L and 221.06±14.35 IU/L and mild fluctuation seen on 28th and 45th day i.e. 163.48±8.70 IU/L and 134.31±6.31 IU/L. Animal of Group II revealed mean serum alkaline phosphatase value as 176.13±16.52 on day 0, later values were found elevated on day 7th and 14th i.e. 206.15±16.18 IU/L and 222.58±14.11 IU/L and mild fluctuation was seen on 28th and 45th day i.e. 165.63±8.63 IU/L and 135.11±6.0 IU/L and was found statistically significant. The osteoblast cells, which are responsible for bone matrix formation and mineralisation, secrete significant quantities of alkaline phosphatase in initial stage and after callus formation is complete, alkaline phosphatase level returns to normal similar findings were observed by Phaneendra *et al.* (2016) [10], Kumar *et al.* (2018) [5]. Similar observations was also recorded by Hegde *et al.* (2007) [4] carried out a comparative evaluation of ALP and concluded significant increase on the operative day as compared to postoperative days.

Table 9: Mean value (±SE) of Alkaline phosphatase (IU/L) at different time intervals

	Before	After	7th day	14th day	28th day	45th day
Group I (n-6)	175.26±16.29	185.53±17.21	204.46±16.25	221.06±14.35	163.48±8.70	134.31±6.31
Group II (n-6)	176.13±16.52	187.06±17.12	206.15±16.18	222.58±14.11	165.63±8.63	135.11±6.06

There was significant difference observed between groups at different interval (p>0.05).

Serum Calcium: In Group I, mean serum calcium concentration ranged from 9.36±0.35mg/dl to 9.8±0.35 mg/dl, whereas in Group II, the range was from 10.33±0.32mg/dl to 10.80±0.30 mg/dl (Table 10). Throughout the observation period, the values showed a non-significant decreasing trend; however, the values fluctuated within normal physiological limits, indicating that the serum calcium values did not change during the fracture healing process. Similar observations were recorded by Kamaljit, 2018 but the decrease in serum calcium reflects a decrease in extracellular calcium, which stimulates the release of calcium-metabolizing hormones according to Komnenou *et al.* (2005) [6].

Table 10: Mean value (±SE) of Serum Calcium at different time intervals

	Before	After	7th day	14th day	28th day	45th day
Group I	9.95±0.367	9.8±0.356	9.53±0.359	9.36±0.357	9.81±0.319	9.96±0.257
Group II	10.80±0.30	10.7±0.32	10.5±0.33	10.33±0.32	10.66±0.29	10.76±0.25

There was non significant difference observed between groups at different interval (p>0.05).

Serum Phosphorus

In Group I, the range of mean serum calcium concentrations was 3.01±0.19 to 3.71±0.18 mg/dl, whereas in Group II, the range was 3.06±0.15 to 3.7±0.15 mg/dl (Table 11). Osteoclastic activity in the early stages of fracture healing, which causes necrotic cell degeneration and resorption of dead bone at the fracture site, may be responsible for the increase in serum calcium and phosphorus and the decrease in calcium uptake from the blood for the mineralization of the callus (Singh, 2015) [13].

Table 11: Mean value (±SE) of Serum Phosphorus (mg/dl) at different time intervals

	Before	After	7th day	14th day	28th day	45th day
Group I	3.71±0.188	3.58±0.185	3.36±0.194	3.23±0.189	3.13±0.180	3.01±0.193
Group II	3.7±0.159	3.6±0.159	3.46±0.158	3.28±0.162	3.16±0.156	3.06±0.156

There was non significant difference observed between groups at different interval (p>0.05).

Conclusion

It is concluded that hemato-biochemical variations did not altered the fracture healing process in all the dogs in both the groups.

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