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RESEARCH ARTICLE

Assessment of fracture healing in dogs based on the evaluation of haemato-biochemical parameters

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ABSTRACT

The purpose of this research is to assess the fracture healing in long bones in dogs based on the haemato-biochemical parameters. All the 18 dogs with long bone fractures were stabilized with intramedullary pinning. Six dogs (Group I) received no grafting material., whereas in Group II and Group III, platelet rich fibrin and CAD-MSCs were inspissated at the fracture site after stabilization. Haematological parameters like haemoglobin (Hb), packed cell volume (PCV), leukocyte, neutrophil, platelets, differential leukocyte count and serum biochemical parameters like calcium, phosphorous and alkaline phosphatase was evaluated in all the animals on 0, 15th, 30th, 45th, and 60th post-operatively. The mean haemoglobin and PCV values in dogs were statistically significant within the groups and also between the groups I & II and I & III. Group III animals showed a significant gradual reduction in leukocyte and neutrophil counts post-operatively compared to groups I, indicating effective immunomodulation and favourable healing dynamics in mesenchymal cell treated group. A significant increase in serum calcium levels was observed in all the groups from 0 day to 30th day, followed by significant decrease on 45th day in group III. However, the calcium values differed significantly within the groups and between the groups I & II and I & III. A significant difference of alkaline phosphatase values was recorded within the groups and between the groups I & II and I &III. Alterations in serum calcium and alkaline phosphatase levels corresponded with osteoblastic activity and mineralization at the fracture site.

Keywords: Dogs, Fracture, Intramedullary pinning, Mesenchymal stem cells, Platelet rich fibrin

INTRODUCTION

Fracture healing in dogs is a complex biological process influenced by fracture characteristics, stabilization method, local biological factors, and postoperative care. Early identification of complications such as infection, inflammation, and delayed bone healing is essential for optimal clinical outcomes. Haematological parameters, including haemoglobin (Hb) concentration, total leucocyte count (TLC), and neutrophil percentage, are routinely assessed as indicators of systemic inflammation and postoperative recovery following fracture stabilization. While fracture union is commonly evaluated through clinical and radiographic methods, biochemical markers allow more sensitive assessment of bone resorption and formation over shorter time intervals.

Serum biochemical markers of bone metabolism particularly alkaline phosphatase, calcium, and phosphorus, provide important insights into osteoblastic activity, mineralization, and the progression of bone healing. Evaluation of these parameters enables objective monitoring of biological events during different phases of fracture repair.

MATERIALS AND METHODS

The study was conducted on dogs presented with long-bone fractures to the Teaching Veterinary Clinical Complex and Department of Veterinary Surgery & Radiology, over a period of 18 months (May 2023 to October 2024). A total of 18 dogs of varying age, sex, and breed were included based on clinical and radiographic confirmation of fractures and divided into three groups of six animals in each group.

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GROUP I:

Animals were subjected to open reduction and the fractured bone was stabilized with stainless steel intramedullary plain pin or positive profile end threaded pinning technique.

GROUP II:

Animals were subjected to open reduction and the fractured bone was stabilized with stainless steel intramedullary plain pin or positive profile end threaded pin and autogenous Platelet Rich Fibrin (PRF) was deposited into the fracture site intra operatively.

GROUP III:

Animals were subjected to open reduction and the fractured bone was stabilized with stainless steel intramedullary plain pin or positive profile end threaded pin and allogenic canine adipose tissue derived mesenchymal stem cells (CAD-MSc) was deposited into the fracture site intra operatively.

All dogs were subjected to detailed pre-operative clinical and orthopaedic examination, which included recording of temperature, pulse, and respiration, assessment of lameness, pain, weight bearing, fracture location and type, presence of crepitus, limb deformity, soft tissue involvement, neurovascular status, and evidence of inflammation or infection at the fracture site.

Pre-operative haematological and biochemical investigations were performed on the day of presentation. Blood samples were collected aseptically for estimation of haemoglobin, packed cell volume, total and differential leukocyte counts, and platelet count, while serum samples were analysed for calcium, phosphorus, and alkaline phosphatase.

The treatment in three groups was evaluated clinically and biochemically on 0, 15th, 30th,45th and 60th post-operative days. Data obtained from clinical, haematological and biochemical evaluations were analysed using appropriate statistical tests (ANOVA). Differences were considered statistically significant at $p < 0.05$.

RESULTS & DISCUSSION

In this study, blood samples were collected on 0, 15th, 30th, 45th, and 60th post-operative days to measure haemoglobin, packed cell volume, total leukocyte count, differential leukocyte count, platelet count and serum levels of calcium, phosphorus, and alkaline phosphatase.

The mean haemoglobin and PCV values in dogs were statistically significant within the groups and also between the groups I & II and I & III. All the values were within the normal physiological range. The progressive rise in haemoglobin values may be attributed to erythropoiesis[1,2,3].

The total leucocyte count in the present study was decreased significantly within the groups and non-significantly between the groups, and the values were within the normal physiological range. This was due to significant reduction of inflammation. Post-operative leukocytopenia may be due to gradual decrease in inflammatory reaction and stress resolution[1]. In this study, higher leukocyte counts on the pre-operative day could be attributed to systemic inflammatory changes following the fracture[3,4,5,6.]

In the present study, the neutrophil count decreased significantly within the groups and also between the groups I & III and non-significant decrease in group I & II, indicating reduced inflammation and infection. The reduced neutrophil count in group II and III compared to group I may be attributed to fact that biomaterials used at the fracture site reduces inflammatory response[1,6].

In our study, group III animals showed a significant gradual reduction in leukocyte and neutrophil counts post-operatively compared to groups I. This can be attributed to the anti-inflammatory effects of mesenchymal stem cells, which shifted the tissue environment from a pro- inflammatory to an anti-inflammatory state[7,8,9], ultimately promoting bone regeneration by reducing the inflammatory phase and thus suppressing the inflammatory response[10,11].

Leucocytosis with neutrophilia is generally observed during the initial stage of inflammation, likely due to fracture-related stress, muscle damage, hematoma, or infection[1,6,12,13]. In our current investigation, all the

Table 1. Biochemical parameters (mean + SE) in the pre- and post-operative period in group I, II & III

S. No.	Parameters	Groups	Time Intervals					
			0th Day	15th Day	30th Day	45th Day	60th Day	Overall mean
1.	Calcium (mg%)	Group I	$8.18^{a}\pm0.19$	$8.55^{ab}\pm0.042$	$9.06^{c}\pm0.07$	$8.70^{bc}\pm0.06$	$8.43^{ab}\pm0.07$	$8.59^{A}\pm0.06$
		Group II	$8.15^{a}\pm0.25$	$9.03^{b}\pm0.08$	$10.05^{c}\pm0.18$	$9.56^{bc}\pm0.12$	$9.25^{b}\pm0.09$	$9.21^{B}\pm0.13$
		Group III	$8.03^{a}\pm0.16$	$9.15^{b}\pm0.08$	$10.53^{d}\pm0.16$	$9.81^{c}\pm0.07$	$9.45^{bc}\pm0.04$	$9.40^{B}\pm0.16$
2.	Phosphorus (mg%)	Group I	$5.15^{a}\pm0.02$	$5.23^{a}\pm0.04$	$5.25^{a}\pm0.02$	$5.21^{a}\pm0.04$	$5.16^{a}\pm0.05$	$5.20^{A}\pm0.01$
		Group II	$5.18^{a}\pm0.03$	$5.26^{a}\pm0.03$	$5.33^{a}\pm0.03$	$5.26^{a}\pm0.04$	$5.20^{a}\pm0.03$	$5.25^{A}\pm0.01$
		Group III	$5.15^{a}\pm0.03$	$5.26^{a}\pm0.03$	$5.36^{a}\pm0.04$	$5.30^{a}\pm0.05$	$5.23^{a}\pm0.04$	$5.26^{A}\pm0.02$
3.	Alkaline phosphatase (U/L)	Group I	$97.50^{ab}\pm1.47$	$101.50^{c}\pm0.22$	$99.33^{bc}\pm0.55$	$98.83^{bc}\pm0.47$	$95.33^{a}\pm0.61$	$98.50^{A}\pm0.50$
		Group II	$97.00^{a}\pm1.15$	$110.50^{b}\pm1.72$	$108.50^{b}\pm1.72$	$106.58^{b}\pm1.24$	$96.00^{a}\pm0.68$	$103.77^{B}\pm1.25$
		Group III	$97.33^{a}\pm1.08$	$115.50^{c}\pm2.04$	$109.16^{b}\pm1.79$	$106.83^{b}\pm0.47$	$96.16^{a}\pm0.47$	$105.00^{B}\pm1.46$

Means bearing different superscript (a, b, c, d) within a row differ significantly $P<0.05$; Means bearing different subscripts (A, B, C, D) within a column differ significantly $P<0.05$

Table 2. Hematological parameters (mean + SE) in the pre and post-operative period in group I, II & III

S. No.	Parameters	Groups	Time Intervals					Overall mean
			0th Day	15th Day	30th Day	45th Day	60th Day	
1.	Haemoglobin (g%)	Group I	$10.10^{a}\pm0.27$	$10.66^{ab}\pm0.25$	$11.01^{b}\pm0.20$	$11.43^{bc}\pm0.20$	$12.06^{c}\pm0.80$	$11.05^{A}\pm0.15$
		Group II	$10.43^{a}\pm0.17$	$11.11^{b}\pm0.09$	$11.65^{c}\pm0.09$	$12.23^{d}\pm10.07$	$12.61^{d}\pm0.09$	$11.61^{B}\pm0.15$
		Group III	$10.38^{a}\pm0.14$	$11.63^{b}\pm0.08$	$12.26^{c}+0.11$	$12.63^{cd}\pm0.08$	$12.95^{d}\pm0.06$	$11.97^{B}\pm0.17$
2.	PCV (%)	Group I	$33.78^{a}\pm0.36$	$34.46^{ab}\pm0.40$	$35.16^{ab}\pm0.42$	$36.10^{bc}\pm0.54$	$37.28^{c}\pm0.70$	$35.36^{A}\pm0.31$
		Group II	$33.71^{a}\pm0.47$	$39.23^{b}\pm0.56$	$40.35^{b}\pm0.59$	$41.20^{b}\pm0.69$	$41.58^{b}\pm0.58$	$39.21^{B}\pm0.58$
		Group III	$34.18^{a}\pm0.44$	$39.73^{b}\pm0.56$	$42.15^{c}\pm0.16$	$42.76^{c}\pm0.21$	$43.38^{c}\pm0.03$	$40.44^{B}\pm0.64$
3.	TLC (x 10^3/µL)	Group I	$18.21^{a}\pm0.24$	$16.73^{b}\pm0.24$	$15.15^{c}\pm0.10$	$14.59^{c}\pm0.17$	$12.83^{d}\pm0.21$	$15.51^{A}\pm0.35$
		Group II	$17.93^{a}\pm0.20$	$15.83^{b}\pm0.25$	$14.48^{c}\pm0.19$	$13.86^{c}\pm0.16$	$12.14^{d}\pm0.26$	$14.85^{A}\pm0.37$
		Group III	$18.06^{a}\pm0.22$	$14.95^{b}\pm0.24$	$13.93^{c}\pm0.12$	$12.90^{d}\pm0.22$	$11.58^{c}+0.17$	$14.29^{A}\pm0.41$
4.	Neutrophil (%)	Group I	$81.66^{a}\pm0.42$	$80.16^{b}\pm0.47$	$78.16^{c}\pm0.30$	$76.33^{d}\pm0.21$	$74.33^{c}\pm0.51$	$78.13^{A}\pm0.50$
		Group II	$82.33^{a}\pm0.42$	$78.66^{b}\pm0.33$	$76.16^{c}\pm0.30$	$74.66^{c}\pm0.33$	$73.00^{d}\pm0.44$	$76.97^{AB}\pm0.62$
		Group III	$82.00^{a}\pm0.57$	$77.33^{b}\pm0.21$	$74.66^{c}\pm0.33$	$73.16^{c}\pm0.03$	$70.66^{d}\pm0.33$	$75.57^{B}\pm0.73$
5.	Lymphocyte (%)	Group I	$13.50^{a}\pm0.22$	$14.66^{ab}\pm0.21$	$15.83^{bc}\pm0.47$	$17.50^{cd}\pm0.76$	$19.33^{c}\pm0.42$	$16.16^{A}\pm0.42$
		Group II	$13.50^{a}\pm0.34$	$15.83^{b}\pm0.16$	$17.00^{b}\pm0.63$	$18.83^{c}\pm0.30$	$19.83^{c}\pm0.47$	$17.00^{A}\pm0.44$
		Group III	$13.50^{a}\pm0.22$	$16.50^{b}\pm0.22$	$17.16^{b}\pm0.79$	$19.50^{c}\pm0.42$	$21.00^{c}\pm0.36$	$17.53^{A}\pm0.51$
6.	Monocyte (%)	Group I	$2.17^{a}\pm0.17$	$2.00^{a}\pm0.26$	$1.83^{a}\pm0.17$	$1.83^{a}\pm0.31$	$1.50^{a}\pm0.22$	$1.86^{A}\pm0.10$
		Group II	$2.33^{a}\pm0.21$	$2.00^{a}\pm0.25$	$1.66^{a}\pm0.33$	$1.50^{a}\pm0.22$	$1.33^{a}\pm0.21$	$1.76^{A}\pm0.12$
		Group III	$2.16^{a}\pm0.16$	$1.83^{ab}\pm0.30$	$1.50^{ab}\pm0.22$	$1.33^{ab}\pm0.21$	$1.16^{b}\pm0.16$	$1.60^{A}\pm0.11$
7.	Eosinophill (%)	Group I	$2.33^{a}\pm0.21$	$2.00^{a}\pm0.36$	$1.83^{a}\pm0.40$	$1.67^{a}\pm0.33$	$1.50^{a}\pm0.22$	$1.86^{A}\pm0.14$
		Group II	$2.50^{a}\pm0.31$	$1.83^{ab}\pm0.30$	$1.50^{ab}\pm0.34$	$1.33^{ab}\pm0.21$	$1.16^{b}\pm0.16$	$1.66^{A}\pm0.14$
		Group III	$2.33^{a}\pm0.21$	$1.66^{ab}\pm0.33$	$1.33^{b}\pm0.21$	$1.16^{b}\pm0.16$	$1.00^{b}\pm0.00$	$1.50^{A}\pm0.12$
8.	Platelets (x10^3/mm^3)	Group I	$240.33^{a}\pm10.89$	$258.50^{a}\pm16.39$	$263.83^{a}\pm5.64$	$261.16^{a}\pm8.60$	$263.33^{a}\pm9.18$	$246.07^{A}\pm6.73$
		Group II	$242.00^{a}\pm11.00$	$268.00^{a}\pm5.39$	$260.66^{a}\pm8.23$	$254.00^{a}\pm7.61$	$266.83^{a}\pm6.90$	$257.77^{A}\pm5.9$
		Group III	$249.00^{a}\pm6.84$	$269.16^{a}\pm4.35$	$257.16^{a}\pm9.87$	$266.16^{a}\pm6.63$	$260.16^{a}\pm2.76$	$258.97^{A}\pm5.8$

Means bearing different superscript (a, b, c, d) within a row differ significantly P<0.05; Means bearing different subscripts (A, B, C, D) within a column differ significantly P<0.05

animals had statistically higher lymphocyte counts on 30th and 60th post operative days compared to pre operative day[1,5], indicating gradual decrease of inflammatory reaction. The higher values of neutrophil counts associated with lower values of lymphocyte counts were noticed in all groups during pre-operative and immediate post-operative days. Cytokines released by macrophages activate the adrenal axis, leading to an increase in glucocorticoid levels[5,6]. This elevation may contribute to the lysis of lymphoid tissues and a reduction in circulating lymphocytes during the initial stage. In the present study, eosinophil and monocyte counts fluctuated within normal physiological limits across all groups, indicating no anaphylactic reactions and confirming the biocompatibility of biomaterials used at the fracture site[1,4,6,13]. Platelet count in the present study fluctuated within the normal range and no significant difference was observed within the groups. The haematological parameters evaluated in the present investigation were summarized in Table 2.

A significant increase in serum calcium levels was observed in all the groups. However, the calcium values differed significantly within the groups and between the groups I & II and I & III. The increase in serum calcium may indicate effective calcium deposition at the fracture site with subsequent formation of new bone with trabeculae[3,14,15,16].

In our study, during the fracture healing there was no significant difference of phosphorus values within the groups or between the groups[14]. The biochemical parameters evaluated in the present investigation are summarized in Table 1.

Serum alkaline phosphatase levels were measured during fracture healing following rigid stabilization. A significant difference of alkaline phosphatase values was recorded within the groups and between the groups I & II and I & III [2,3,12,16]. There were increased alkaline phosphatase levels following fracture immobilization in dogs[17]. Alkaline phosphatase, present in osteoblast membrane, played a role in the synthesis and mineralization of the bone matrix and

its raised levels might be attributed to increased osteoblastic activity to cause bone formation during fractured bone repair and thus formation of new bone matrix[18,19,20].

CONCLUSION

Serial evaluation of haematological and biochemical parameters enabled objective monitoring of inflammatory status and bone metabolic activity during fracture healing in dogs. A significant postoperative decline in leukocyte and neutrophil counts, particularly in mesenchymal stem cell–treated animals, indicated effective immunomodulation and favourable healing dynamics. Alterations in serum calcium and alkaline phosphatase levels corresponded with osteoblastic activity and mineralization at the fracture site. These findings support the utility of haemato-biochemical markers for assessing fracture repair and the biological efficacy of regenerative interventions in canine long-bone fractures. All the animals of IIIrd group exhibited excellent functional limb outcomes with no lameness by the end of 60th post operative day.

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