



Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

HAEMATO-BIOCHEMICAL, RADIOGRAPHIC AND CLINICAL OUTCOME IN HEALING OF FEMORAL FRACTURE WITH RETROGRADE INTRAMEDULLARY PIN IN CONJUNCTION WITH DEMINERALIZED BONE MATRIX IN DOGS

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Received – February 26, 2017; Revision – April 21, 2017; Accepted – May 01, 2017

Available Online – May 12, 2017

DOI: [http://dx.doi.org/10.18006/2017.5\(2\).201.207](http://dx.doi.org/10.18006/2017.5(2).201.207)

KEYWORDS

Demineralized bone matrix

Femoral fracture healing

Haemato –biochemical

Radiographic

Clinical outcome

ABSTRACT

The study was undertaken to evaluate the haemato-biochemical, radiographic and clinical outcome of femoral fracture healing in dogs. The dogs were treated with open reduction and internal fixation (ORIF) with retrograde intramedullary pin with or without full circlage wire (Group I) and ORIF with retrograde intramedullary pin with or without full circlage wire along with incorporation of demineralized bone matrix (Group II) at the fracture site. The haemato-biochemical, radiographic and clinical outcome assessment were done on 0, 21st, 45th and 60th day. The haemato-biochemical parameters studied were alkaline phosphatase, creatinine kinase, serum calcium, total erythrocyte count, total leucocyte count, haemoglobin, packed cell volume, lymphocyte, monocyte, thrombocyte, mean platelet volume, mean corpuscular volume and mean corpuscular haemoglobin. Modified Thomas splint was applied post operatively till 21st day; dogs started walking from 3rd day onwards. The haematological, radiographic and clinical outcome in the different time period and between the groups showed non-significant minor variation during the period of study. The biochemical activity of alkaline phosphatase was better in group II. On 60th day radiograph showed complete bridging of fracture end with callus in both the group except for one animal of group II. Based on better biochemical activity of alkaline phosphatase in group II and similar haematological, radiographic and clinical outcome it can be concluded that demineralized bone matrix can augment the healing of femoral fracture.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

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1 Introduction

Femur fracture accounts for highest percentage of fracture occurrence in small animals (Simon et al., 2010; Kushwaha et al., 2011). The objective of fracture treatment is to achieve fastest possible healing and enable the patient to early ambulation (Shahar, 2000). Intramedullary pinning is most widely used for internal fixation because it require minimal exposure of the fracture site, speed of insertion, low cost, require less technical expertise and equipment, least sophisticated, resist bending force in all directions, give stronger repair to the fracture site in early stage of healing and its strength is related to its diameter (Newton & Nunamaker, 1985; Denny & Butterworth, 2000). Fracture healing is a complex physiological process it involves many types of cells, biochemical regulating factors and expression of several thousand genes (Eihorn, 1998). Demineralized bone matrixes are obtained either from bovine, porcine or human cadaver. It is prepared by pulverization into consistent size followed by decalcification with hydrochloric acid. As a result of decalcification mineral component are lost but retains the Type I collagen, non-collagenous proteins, osteoinductive growth factors including varying concentration of bone morphogenetic proteins (BMPs), growth differentiation factors and other transforming growth factors (TGF-b1, TGF-b2, TGF-b3)(Kale & DiCesare,1995; Urist et al.,1983;Schwartz et al., 2011; Gruskin et al.,2012; Holt & Grainer, 2012). The present study was undertaken to evaluate the haemato-biochemical, radiographic and clinical outcome of femoral fracture healing in dogs treated with open reduction and internal fixation (ORIF) with retrograde intramedullary pinning in conjunction with demineralized bone matrix.

2 Materials and methods

Twelve dogs irrespective of their age, sex, breed, body weight with history of road traffic accident, fall from height and being hit by iron bar or any other form of trauma presented to the Department of Surgery and Radiology, College of Veterinary Science, Khanapara, Assam Agricultural University were taken into study. These dogs were clinically evaluated, administered preliminary medicinal treatment, care and were subjected for confirmatory diagnosis by radiograph. After confirmatory diagnosis for femoral fracture these dogs were divided into two groups comprising of six animals in each group for treatment as-

Group I- Open reduction retrograde intramedullary pinning with or without full circlage wiring

Group II- Open reduction retrograde intramedullary pinning with or without full circlage wiring along with incorporation of demineralized bone matrix (DBM) in the form of paste at the fracture ends

The dogs were kept off fed for 12 hours and off water for 6 hours respectively. Premedication was done with atropine sulphate at the dose rate of 0.04mg/kg bwt intramuscularly 15 minutes prior to administration of xylazine hydrochloride at the dose rate of 1mg/kg bwt intramuscularly. Then a combination of diazepam at the dose

rate of 0.3 mg/kg bwt and ketamine hydrochloride at the dose rate of 5 mg/kg bwt intravenous was used as an induction agent and for maintenance of anaesthesia throughout the surgery as supplemental dose. Retrograde intramedullary pinning covering 60-75% of the medullary cavity was done and treatment was rendered as per the groups. The surgical wounds were closed in routine manner. External immobilization was done with Modified Thomas splint for three weeks which was changed at regular interval of one week. The post-operative treatment regime comprised of inj. Ceftriaxone-sulbactam at the dose rate of 15mg/kg b.i.d for 5 days, inj gentamicin at the dose rate of 5mg/kg b.i.d. bwt intramuscularly for 5 days, inj. butorphanol at the dose rate of 0.2mg/kg bwt intramuscularly b.i.d for 5 days and fluid therapy with dextrose in normal saline for 5 days. Three milliliter (3ml) of blood was collected prior to surgery on 0 and on 21st, 45th and 60th day for biochemical, haematological study and plain radiograph was taken on same intervals. The dogs were clinically evaluated on 21st, 45th and on 60th day. The biochemical parameters studied were Alkaline phosphatase (ALP), Creatinine kinase (CK), Serum Calcium (Ca). The alkaline phosphatase was estimated as per the p-nitrophenyl phosphate method from the commercially available kit using UV visible spectrophotometer and expressed as U/L. Creatinine kinase was estimated as per IFCC method from commercially available kit using UV spectrophotometer and expressed as U/L. Serum calcium was estimated by the procedure cresolphthalein complex one method using UV spectrophotometer and expressed as U/L. Haematological parameters studies included Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Haemoglobin (Hb), Packed Cell Volume (PCV), Lymphocyte (Ly), Monocyte (M), Thrombocyte (T), Mean Platelet Volume (MPV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and were estimated using haemoautoanalyzer.

Statistical analysis was performed by Student t- test and Analysis of variance (ANOVA) using Statistical package of SAS enterprise guide 4.2 with level of significance at 5%.

3 Results and Discussion

The alkaline phosphatase (ALP) in group I, showed highly significant difference ($P < 0.01$) on 21st days and significant difference ($P < 0.05$) on 60th days (Table 1). While in case of group II highly significant difference ($P < 0.01$) was observed on 21st, 45th days and significant difference ($P < 0.05$) on 60th days (Table 1). Highly significant difference ($P < 0.01$) was observed on 45th and significant difference ($P < 0.05$) on 60th days between the group I and group II (Table 1). Higher values were observed in group II on 21st, 45th and 60th day. Increase in osteoblastic activity indicates normal fracture healing. Large amount of alkaline phosphatase is released by the osteoblast, these alkaline phosphatase leads to formation of bone matrix and its mineralization (Leung et al., 1993). Alkaline phosphatase is supposed to increase the concentration of local inorganic phosphate or neutralize inorganic pyrophosphate (Seropoulos & Anagnostopoulos, 1997; Volpin et al., 1998).

Table 1 Value of various haematological and biochemical parameters in studied dogs

Parameters	Treatments	Periods (Days)			
		0	21	45	60
Total Erythrocyte Count	GROUP I	5.27 ±0.15 ^a _A	5.68 ±0.12 ^a _B	5.54 ±0.14 ^a _B	5.98 ±0.15 ^a _B
	GROUP II	4.09 ± 0.36 ^b _A	5.02 ± 0.47 ^a _B	5.65 ± 0.21 ^a _C	4.66± .79 ^b _A
Total Leucocyte Count	GROUP I	30.88 ± 2.83 ^a _A	22.75 ±2.30 ^a _A	15.80 ± 3.01 ^a _B	15.60± .51 ^a _B
	GROUP II	25.07 ± 3.24 ^b _A	15.68 ±0.75 ^b _B	15.05 ±0.74 ^a _B	12.65±1.41 ^a _B
Haemoglobin	GROUP I	9.45 ± 0.27 ^a _A	10.37 ±0.22 ^a _B	10.78 ±0.34 ^a _B	12.22 ±0.20 ^a _C
	GROUP II	8.98 ± 0.87 ^a _A	9.58 ±0.97 ^a _A	10.20 ±0.56 ^a _B	9.00 ±1.53 ^a _A
Lymphocyte	GROUP I	7.44±1.61 ^a _A	7.44 ±1.61 ^a _A	5.19±1.18 ^a _B	4.26±1.14 ^a _B
	GROUP II	3.53 ± 0.32 ^c _A	3.53±0.32 ^c _A	2.92±0.34 ^c _A	1.23±0.35 ^c _B
Monocyte	GROUP I	2.07±0.57 ^a _A	1.99±0.12 ^a _A	1.52±0.17 ^a _A	1.07±0.14 ^a _B
	GROUP II	0.53 ±0.06 ^c _A	0.61±0.08 ^c _A	0.58 ±0.08 ^b _A	0.31±0.07 ^b _B
Thrombocyte	GROUP I	177.17±22.88 ^a _A	177.17±22.88 ^a _A	95.50±16.06 ^b _B	77.50±7.68 ^a _C
	GROUP II	163.33±42.45 ^a _A	163.33±42.45 ^a _A	125.33±64.14 ^c _B	47.33±13.02 ^c _C
MPV	GROUP I	8.22±0.31 ^a _A	8.35±0.25 ^a _A	8.38±0.25 ^a _A	8.28±0.35 ^a _A
	GROUP II	7.48±0.39 ^a _A	7.03±0.48 ^b _A	7.30±0.62 ^a _A	6.74±1.40 ^b _B
MCV	GROUP I	62.73±1.71 ^a _A	61.75±1.86 ^a _A	59.67±1.16 ^b _B	59.60±0.31 ^a _B
	GROUP II	64.47±0.32 ^a _A	61.88±0.39 ^a _A	63.05±0.35 ^a _A	58.33±3.48 ^a _B
MCH	GROUP I	21.37±0.53 ^a _A	19.94±0.51 ^a _B	23.70±2.75 ^a _A	18.71±0.71 ^a _B
	GROUP II	18.58±0.56 ^b _A	19.57±0.72 ^a _A	19.33±0.27 ^b _A	19.70±0.13 ^a _A
ALP	GROUP I	84.39±2.71 ^a _A	190.88±3.61 ^a _C	76.85±6.39 ^a _A	60.70±7.39 ^b _B
	GROUP II	59.71±11.86 ^a _A	204.48±9.39 ^a _D	155.65±19.54 ^c _C	109.27±19.27 ^a _B
Creatinine Kinase	GROUP I	184.73±6.59 ^a _A	106.87±14.42 ^a _B	56.00 ±3.23 ^a _C	23.08±1.07 ^a _D
	GROUP II	212.21±22.31 ^b _A	94.57±5.98 ^a _B	47.72± 1.29 ^a _C	20.39±0.80 ^a _D
Serum Calcium	GROUP I	4.62±1.32 ^a _A	4.35±1.31 ^a _A	7.97±0.75 ^a _B	9.70 ±1.18 ^a _C
	GROUP II	5.69 ±0.31 ^a _A	4.80±0.44 ^a _A	7.98±0.20 ^a _B	8.87 ±0.06 ^a _C

Value given in table represents MEAN ± SE value; *Mean bearing similar superscript between the groups do not differ significantly (a, b, c); **Mean bearing similar subscript between the periods within the group do not differ significantly (A, B, C, D)

DBM is osteoinductive and osteoconductive biomaterial it might facilitate new bone formation by allowing the cells in the local environment to undergo phenotypic conversion to osteoprogenitor cells and might provide mechanical support for vascular and bone in growth (Urist et al., 1983; Kale & Di Cesare, 1995; Albrek & Johansen, 2001; Pieske et al., 2009; Schwartz et al., 2011; Gruskin et al., 2012; Holt & Grainer., 2012). The demineralization process of DBM by hydrochloric acid (0.6ml/litre) might enhance the bone morphogenic protein to stimulate local undifferentiated mesenchymal cell to transform into osteoblast (Riley et al., 1996). The greater elevation of alkaline phosphatase in group II in comparison to group I (Table.1) dogs indicates more osteoblastic activity aided by demineralized bone matrix. Fracture healing can be assessed well with alkaline phosphatase however bone specific isoenzyme of alkaline phosphatase (BALP) is specific (Leung et al., 1993). The finding correlates with previous work (Kumar & Ramakrishna, 1996).

In group I, highly significant difference (P<0.01) was reported in creatinine kinase on 0 day, 21st, 45th and 60th day (Table 1). While in case of group II significant difference (P<0.05) was noticed on 21st day and highly significant difference (P<0.01) on 45th and 60th day (Table 1). Significant difference (P<0.05) was observed on 0 day

between the group I and group II (Table 1). The initial increase in the level of creatinine kinase within the physiological limit may be attributed to the muscle trauma, swelled area due to trauma, displaced bone fragment, direct trauma leading to fracture (Larsson & Linden, 1981; Onio et al., 1989) and stress during the fracture healing phase. The external immobilization in the form of Modified Thomas Splint might have attributed to the stress to the animals, as after its removal on 21st day the creatinine kinase level decreased.

Calcium showed significant difference in group I (P<0.05) on 45th day and highly significant difference (P<0.01) on 60th day (Table 1). In group II significant difference (P<0.05) was observed on 45th day and highly significant difference (P<0.01) on 60th day (Table 1). Comparison between these two groups revealed no difference. The decrease in the level of serum calcium can be attributed to increased urinary excretion after traumatic bone injury (Kumar et al., 1992). The finding correlates with finding of previous study (Struck et al., 1969; Rani & Ganesh, 2003) however contrary to findings of Kumar & Ramakrishna (1996); Howard et al. (1945). The increasing trend from 45th day onward of serum calcium in present study was indicative of normal union (Verma & Singh, 2003).



Fig. 1 0 day (Group I)



Fig. 2 0 day (Group II)



Fig. 3 21st day (Group I)



Fig. 4 21st day (Group II)



Fig. 5 45th day (Group I)



Fig. 6 45th day (Group II)



Fig. 7 60th day (Group I)



Fig. 8 60th day (Group II)

Figure 1 Showing lateral radiograph on 0 day of complete metaphyseal fracture in a 2 year old, male, Dalmatian dog (Group I); Figure 2 Showing lateral radiograph on 0 day of complete multiple diaphyseal fracture in a 4 year old, male, Pomerian dog (Group II); Figure 3 Showing lateral radiograph on 21st day after intramedullary pin fixation of complete metaphyseal fracture in a 2 year old, male, Dalmatian dog (Group I); Figure 4 Showing lateral radiograph on 21st day after intramedullary pin fixation and full circlage wire fixation in a 4 year old, male, Pomerian dog (Group II); Figure 5 Showing lateral radiograph on 45th day after intramedullary pin fixation of complete metaphyseal fracture in a 2 year old, male, Dalmatian dog (Group I); Figure 6 Showing lateral radiograph on 45th day after intramedullary pin fixation and full circlage wire fixation in a 4 year old, male, Pomerian dog (Group II); Figure 7 Showing lateral radiograph on 60th day after intramedullary pin fixation of complete metaphyseal fracture in a 2 year old, male, Dalmatian dog (Group I); Figure 8 Showing lateral radiograph on 60th day of complete multiple diaphyseal fracture after intramedullary pin fixation and full circlage wire fixation in a 4 year old, male, Pomerian dog (Group II).

Total Erythrocyte Count in group I revealed significant difference ($P < 0.05$) on 21st and 60th day (Table 1). In group II a significant difference ($P < 0.05$) was observed on 21st day and highly significant difference ($P < 0.01$) on 45th day (Table 1). Significant difference ($P < 0.05$) was noted on 0 and 60th day between the group I and group II (Table 1). The difference between the groups on 0 day might be because of the blood loss in group II dogs as two animals had compound fracture. The TEC in group II did not reach the normal physiological level this can be attributed to the ability of demineralized bone matrix of pooling erythrocytes as they form vascular bed around the fracture site (Pieske et al., 2009; Albrek & Johanssen, 2001) during the fracture healing stage as by 60th day the value in group I animal reached normal physiological limit.

In group I on 45th day and 60th day significant difference ($P < 0.05$) was observed in total leucocyte count (Table 1). In group II significant difference ($P < 0.05$) was observed on 0, 21st, 45th and 60th day (Table 1). Significant difference ($P < 0.05$) was observed on 0 and 21st day between the group I and group II (Table 1). The increase in the level of TLC in both the groups on 0 day can be attributed to the normal response of body to trauma (Aithal, 1998). The increase in TLC in group I on 21st day can be because of stress to the dogs due to presence of external immobilization or confining the animal to avoid unnecessary movement. Findings of present study correlates with findings of Aithal (1998). The authors in the

present study ruled out any infection as none of the animal in the present study developed osteomyelitis and were apparently healthy.

Haemoglobin estimation revealed significant difference in group I ($P < 0.05$) on 21st, 45th day and highly significant difference ($P < 0.01$) on 60th day (Table 1). In group II highly significant difference ($P < 0.01$) was observed on 45th day (Table 1). Non-significant difference was observed between the group I and group II. The group II dogs did not attained the normal physiological level which indicates that the dogs were anaemic. The perusal of literature revealed non availability of literature on role of DBM on haemoglobin. Whether the decrease in haemoglobin is due to DBM or any underlying disease is not clear as the blood were analyzed for haemoprotozoan and rickettsial disease and found negative.

Significant difference ($P < 0.05$) was observed in Lymphocyte in group I on 45th and 60th day. In group II significant difference ($P < 0.05$) was observed on 60th day. Highly significant difference ($P < 0.01$) was observed between the groups on 0, 21st, 45th and 60th day (Table 1). The level of lymphocyte in both the groups throughout the period of study was below the physiological level. Aithal (1998) recorded reduction in the level of lymphocyte till 3rd day of study and concluded that it is normal response of body in early operative days. In the present study it can be attributed to the pain and flight – fright response of the body on 0 day but decrease till 21st day can be attributed to stress, anxiety on the dogs due to the presence of external immobilization. However the level of

lymphocyte below physiological limit from 45th to 60th day in both groups remains unexplainable.

Monocyte showed significant difference ($P < 0.05$) between group I and II on 45th and 60th day however highly significant difference ($P < 0.01$) was observed on 0, 21st days (Table 1). In group I and in group II significant difference ($P < 0.05$) was observed on 60th (Table 1). In group I increase in the level of monocyte on 0, 21st day may be attributed to stress on the animal due to trauma, tissue damage, and external immobilization.

In group I significant difference ($P < 0.05$) was noted in Thrombocyte on 45th day while highly significant difference ($P < 0.01$) on 60th day (Table 1). In group II significant difference ($P < 0.05$) was observed on 45th day and highly significant difference ($P < 0.01$) was observed on 60th day. Highly significant difference ($P < 0.01$) was observed on 45th and 60th day between the group I and group II. The values were within normal range in both the group till 21st day and up to 45th day in group II but below normal in group I on 45th and 60th day, and in group II on 60th day. The blood samples were analyzed for haemoprotozoan and rickettsial disease but were found to be negative and the dogs did not have history of any poisoning.

Mean Platelet Volume (MPV) revealed non-significant difference in group I. Significant difference ($P < 0.05$) was observed on 60th day in group II (Table 1). Significant difference ($P < 0.05$) was observed on 21st and 60th day between the group I and group II (Table 1). The changes were within physiological limit.

Mean Corpuscular Volume (MCV) revealed significant difference ($P < 0.05$) in the group I on 45th and 60th day. On 60th day significant difference ($P < 0.05$) was observed in group II. Between the group I and group II significant difference ($P < 0.05$) was observed on 45th day. The MCV value in both the group was below the normal physiological value from 0 day and had a declining trend till 60th day. There are no available literatures describing about role of DBM on MCV. The finding indicates that the dogs were having anaemia in both the groups.

In group I significant difference ($P < 0.05$) was observed in mean corpuscular haemoglobin (MCH) on 21st and 60th day (Table 1). In group II non-significant difference was observed. Significant difference ($P < 0.05$) was observed between the group on 0 and 45th day (Table 1). There are no available literatures describing role of DBM on MCH however findings indicates that the dogs were having anaemia in both the groups.

The preoperative radiograph in both the group revealed clear fracture end (Figure 1, 2). Radiograph taken post operatively in both the groups showed proper reduction, good alignment of both the fractured ends; with a clear fracture line in between the bone fragments without any angulations or displacement of the fragment as the small fragment were immobilized using full circlage wire. On 21st day the radiograph showed irregular border and feathery appearance, periosteal callus around the fracture site with little formation of callus at the site of fracture in both the groups (Figure

3, 4). On 45th day radiograph revealed intense callus almost completely bridging the fracture end (Figure 5) in both the groups except one patient of group II with comminuted fracture having three fragments (Figure 6). On 60th day the fracture was completely bridged with callus (Figure 7) except in one patient of group II (Figure 8). The non-complete bridging of callus by 60th day might have resulted due to mechanical obstruction by full circlage wire during healing phase (Figure 8). Two patients had pin migration by 60th day comprising one animal from each group. The intramedullary pins were removed in both the groups on 60th day.

The animals of both the groups started walking from the third day onwards. The Modified Thomas splint was changed at one week interval up to third week. It was done to avoid skin damaged because of cotton padding as it causes increase in temperature to the local tissue and increased tissue perspiration. As increase in humidity and tissue perspiration can moisten the cotton and it can become a good media for fungal and bacterial growth. It also aided in inspection of wound and also letting the animal bear its weight on the affected limb and mobilizing the limb for a short period of time. After removal of Modified Thomas Splint on third week, the animals were able to completely bear weight and walk normally with the affected limb though stiffening of gait was observed in 3 animals. These were corrected with physiotherapy session for thrice a week. However one animal of group II was partially weight bearing and hence the Modified Thomas splint was applied up to 45th day. In this animal the callus did not bridge by 60th day and had limb shortening (Figure 8).

Conclusion

The present study revealed that in both the group haematological, radiographic and clinical outcome were similar. The alkaline phosphatase showed better biochemical activity in group II. Therefore femoral fracture repaired with ORIF along with incorporation of demineralized bone matrix may aid in healing of femoral fracture during the biochemical phases of fracture healing.

Ethical Approval

The present study was approved by Institutional Animal Ethics Committee.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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