



Clinical Evaluation of Demineralized Bone Matrix Allograft in Femur Fracture in Dogs

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ABSTRACT

Eight clinical cases of dogs having femur fracture, were divided into two groups, A and B with four dogs (n=4) in each group. Group A animals were treated with intramedullary pinning (IMP) alone and group B were treated using IMP alongwith demineralized bone matrix (DBM) implantation at the fracture site. The efficacy of healing was evaluated on the basis of clinical evaluation, haemato-biochemical and radiographic parameters on the day of admission (day 0), followed by 7th, 15th, 30th, 45th and 60th post-operative days. The weight bearing was observed to be better in dogs of group A. Swelling was completely absent after 15th post-operative day in dogs of both the groups. The mean values of Hb, PCV, TLC and DLC showed a non-significant variation on subsequent post-operative days in both the groups. There was a significant increase in serum calcium up to 30th post-operative day and thereafter, followed a decreasing trend. The serum alkaline phosphatase values showed non-significant variation in group A while in group B animals significant increase observed on 15th, 30th and 45th post-operative day. Radiographically, the dogs of group B showed better radiographic union of fracture evidenced by early disappearance of fracture line than those of group A.

Keywords: Allograft, DBM, Dog, Femur fracture, intramedullary pinning

Fracture of long bones has been the most important orthopaedic problems encountered in dogs, femur having the highest incidence followed by tibia, radius-ulna and humerus (Aithal *et al.*, 1999; Simson *et al.*, 2010). Most of the time, healing of these fractures is associated with various complications like a delayed union, non-union and mal-union. Bone grafting procedure is the second most commonly performed procedure in human beings after the blood transfusion to address the fracture healing complications. Among bone graft materials, autograft is considered to be the gold standard or ideal graft material. But, it is also associated with complications like limited blood supply, donor site morbidity and more painful. Allografts are the alternative to autograft and are recovered from living or deceased donors. Demineralized Bone Matrix (DBM) is an allograft bone that has the inorganic mineral removed, leaving behind the organic collagen matrix. It is prepared by pulverization into consistent

size followed by demineralization using inorganic acid leaving behind the collagen, non-collagenous proteins, osteoinductive growth factors and growth differentiation factors (Urist *et al.*, 1983; Schwartz *et al.*, 2011).

DBM provides no structural strength and is primarily used in the structurally stable environment. It revascularizes quickly and acts as suitable carrier for autologous bone marrow. The biological activity of demineralised bone matrix is presumably attributable to proteins and various growth factors present in the extracellular matrix and is made available to the host environment by demineralization process. In the view of above, the present study was planned to evaluate the demineralized bone matrix in the healing of femur fracture in dogs.

MATERIALS AND METHODS

Eight cases of femur fracture presented to teaching

veterinary clinical complex, SKUAST-J, were randomly divided into two groups viz. A and B, with each group consisting of 4 animals. After recording of preliminary data related to signalment, clinical examination, haemato-biochemical evaluation and radiographic examination, Group A animals were subjected to IM pinning alone while group B animals were subjected to IM pinning alongwith application of demineralised bone matrix (DBM) at fracture site (Fig. 1g).

Clinical evaluation (lameness, weight-bearing, pain on palpation, swelling etc of the affected limb) was done on day 0 (the day of admission) and post-operatively on day 7, 15, 30, 45 and pin removal. Radiographic evaluation was done on day 0 and immediately after the fracture fixation to note the reduction and pin fixation status and on 7th, 15th, 30th, 45th and 60th post-operative days to assess the healing status of the fracture in terms of callus formation and union of the fractured bone fragments. Haemato-biochemical evaluation (Hb, TLC, DLC, PCV, Calcium, Phosphorus, Total Protein, Alkaline Phosphatase and Albumin) were carried out on day 0, 7th, 15th, 30th, 45th and on 60th day.

Demineralized bone matrix was prepared from the long bone of a dog brought to us for limb amputation following the protocol followed by Tunio *et al.*, 2012 (Fig. 1a to 1f).

RESULTS AND DISCUSSION

In the present study, eight clinical cases with femur fracture of either sex, with age varied between 8 months to 2 years involving right limb in 5 cases and left limb in 3 cases were included for clinical evaluation of demineralized bone matrix allograft in fracture healing.

The pain, lameness and swelling score were observed to be higher on a preoperative day but reduced significantly ($P < 0.05$) on successive post-operative days in both groups. On the 60th post-operative day these scores were found lower in group B animals than group A animals.

The high pain, lameness and swelling score on pre-operative day might be due to the inflammatory reaction initiated by the trauma, which gradually subsided at the end of the study in both the groups leading to significant reduction in pain, lameness and swelling. Further, the lower score in group B animals could be due to additional support and promotion of the healing process by DBM leading to faster healing. The findings of Jani (2013) are in concurrence with present observations.

The weight bearing score found to be significantly increased in both the groups on subsequent post-operative days. This might be due to a decrease in pain due to the healing of fractured bone. The weight bearing on affected



Fig. 1: Preparation of demineralized bone matrix from dog long bones. (a) Collection of long bone (tibia) for preparation of DBM; (b) Manual removal of cortex; (c) Defatting of frozen bone; (d) Decalcification in 0.6M HCl, (e) Lyophilization in freeze dryer; (f) Prepared DBM for use in the study; (g) Intraoperative application of DBM crystals at fracture site

limb was observed to be better in group B animals as compared to group A animals but it was statistically non-significant. Jani (2013) also observed gradual increases in weight bearing score in the successive post-operative period, when compared to day zero or the day of admission.

The haematological parameters Hb, PCV, TLC and DLC showed non-significant changes on subsequent post-operative days. The non-significant changes in TLC and DLC also indicate that the allograft did not elicit any untoward reaction either locally or systemically. Singh *et al.* (2017) also reported non-significant haematological variations during femoral fracture healing following retrograde intramedullary pinning in conjunction with demineralized bone matrix.

The levels of calcium showed an increasing trend upto 30th post-operative day in both the groups thereafter a decreasing trend noticed upto 60th post-operative day in both the groups.

The initial increase in serum calcium can be attributed to the oral supplementation of calcium and Vitamin D₃ to the affected dogs. Further, it indicates that the process of soft callus mineralization has yet to start. After an initial increase there was a decreasing trend observed in both groups this could be due to the utilization of calcium in mineralization of soft callus at fracture site. In contrary to our findings Rani *et al.* (2012) observed high levels of serum calcium on subsequent post-operative days during fracture healing. Dwivedi *et al.* (2009) observed non-significant variation in serum calcium during compound fracture healing of long bones in subsequent post-operative period.

In group A, the levels of phosphorus were lower on the day of admission and showed a non significant increase upto 15th post operative day, followed by a significant decrease upto 60th post-operative day. In group B, the levels of serum phosphorus showed a significant increase upto higher normal levels on 30th post-operative day and then decreased significantly ($P < 0.05$). The levels of serum phosphorus showed a significant variation on 30th and 45th post-operative days between the groups.

The slight hyperphosphatemia within normal range can be attributed to the collagen synthesis and increased osteoblastic stimulation at the fractured site as also reported by Manjunath (2010). Dwivedi *et al.* (2009) also

observed that the increased osteoblastic stimulation and collagen synthesis at the fracture site might be a reason for increase in the phosphorous level. The increase in the level of phosphorous might also be attributed to the necrotic disintegration of cells at the site of fracture (Singh *et al.*, 1976).

The values of serum total protein showed a significant increase on 30th post-operative day followed by a decrease in serum levels on 60th post operative day in dogs of both groups A and B.

Pain and lameness in early post-operative days might have caused anorexia leading to decreased levels of total protein. However, the levels of total protein remained around the base value. The variation in total protein of individual dogs may have resulted in the variation in total proteins of different groups. The plasma level of total protein is influenced by the appetite status, dehydration and chronic starvation. Decreased level of total plasma protein is seen in anorexia and chronic starvation, whereas dehydration could lead in to high level of total protein (Kaneko *et al.*, 1997).

In Group A, the level of serum alkaline phosphatase showed a non-significant ($P > 0.05$) variation throughout the post-operative period while in group B animals there was a significant increase in level of serum alkaline phosphatase observed on 15th, 30th and 45th post-operative day thereafter it starts reducing. However, the values fluctuated within the normal physiological range.

The increase of serum alkaline phosphatase may be attributed to chondroblastic proliferation to cause bone formation. The maximum contribution is from the periosteum of the fractured bone, which is a rich source of alkaline phosphatase. Higher levels, although within the normal reference range, of alkaline phosphatase on day zero might be due to exuberant proliferation of fibrous tissue at the fracture site following bone injury, proliferation of maturing osteogenic cells and active osteoblast cells and the formation of bone matrix (Umashankar and Ranganath, 2008). Further, the increased activity of serum alkaline phosphatase in group B animals indicates increased activity of osteoblast leads to rapid formation of bone matrix and its mineralization (Leung *et al.*, 1993).

The mean \pm SE values of serum albumin showed a significant increase on subsequent post-operative days in

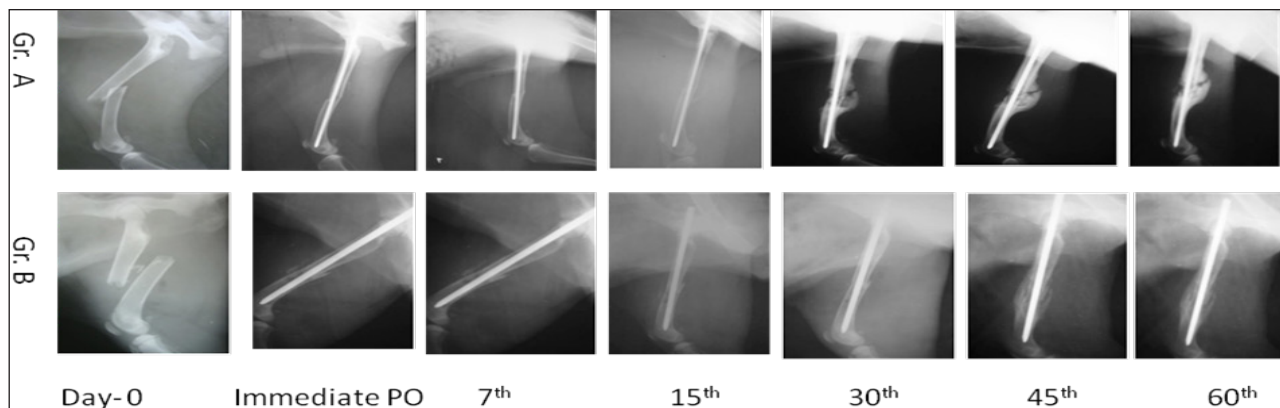


Fig 2: X-ray of group A & group B animal on pre-operative day (0 day), immediate post-operative and on 7th, 15th, 30th, 45th and 60th day. On 30th post-operative radiograph in group A showed large non-organized, non-bridging callus, while in group B fracture line was not visible. On 45th and 60th day radiograph in group B organized periosteal bridging callus seen

group B animals. The levels decreased significantly on 60th post-operative day.

As albumin is a negative acute phase protein, the initial lower levels are suggestive of inflammatory reaction and decreased appetite. Furthermore, the levels gradually increased from 7th post operative day onwards indicating a return to normal appetite. The results were in agreement with the results of Meyer *et al.* (2003).

The pre-operative radiograph in both the groups revealed clear fracture ends. Immediate post-operative radiograph (antero-posterior and lateral views) revealed perfect alignment and proper fixation in three dogs in group A and two dogs in group B. Rest of the cases showed a slight mal-alignment. This may be due to inability of intramedullary pin to resist the torsional forces acting on fractured fragments and fracture configuration. This was in accordance with the findings of Raghunath and Singh (2008). On 30th post-operative day large unorganized callus noticed in group A animals and fracture line was clearly visible, while in group B animals the fracture line was not visible with no any periosteal callus. Further, on 45th and 60th day radiograph showed biologically active large unorganized periosteal callus seen in group A animals while in group B animals bridging periosteal callus almost completely bridging fracture line was seen (Fig. 2).

On 60th post operative radiograph, the fracture gap was obliterated completely. The bones were in good apposition and showed signs of excellent healing in group B, where DBM was used, as compared to 0 day.

On the basis of aforesaid findings we may conclude that demineralized bone matrix allograft promoted in fracture healing without any side effect during femur fracture management in dogs.

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