

## Bone Healing in Femoral Fracture of White Rat with Intramedullary Wire Fixation and Additional Medullary Bone Marrow

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### ABSTRACT

**Introduction.** Fracture treatment and bone healing is a complicated process and interesting topic. There are a lot of researches in this matter until now. Fracture fixation with additional autogenous bone marrow is well known to have good fracture healing. Bone marrow contains progenitor cells that can be used to facilitate bone healing. Fracture treatment with intramedullary nailing combine with bone marrow from iliac crest or femoral head has shown to have better healing. The purpose of this study was to evaluate bone healing on the fracture site by giving the bone marrow from medulla.

**Materials and methods.** In the present study, we explored bone healing in femoral fracture of *Sprague dawley* rat with intramedullary wire fixation and the application of medullary bone marrow as much as 0.5 to 1 cc from femur. The effect of given bone marrow in fracture healing was evaluated from callus formation (callus diameter, callus volume) and alkaline phosphatase level. Subjects were divided into 2 group, namely study group and control group. Each group consists of 20 white rats. The control group was treated with intramedullary wire while the study group was treated with medullary bone marrow in addition to intramedullary wire. Observation was followed until 30 days. During that time, both groups were given same environment including cage, wound treatment, food intake, water intake and temperature). Callus diameter and volume was evaluated with radiological and alkaline phosphatase level was measured from blood serum.

**Results.** Callus diameter in study group was 34.1% larger than control group ( $p < 0.01$ ). Callus volume in study group was 2.02 times larger than control group ( $123.77 \text{ mm}^3$  compared to  $41.23 \text{ mm}^3$ ;  $p < 0.01$ ). Alkaline phosphatase in study group was 23.63% larger than control group ( $30.56 \text{ IU/L}$  compared to  $24.72 \text{ IU/L}$ ;  $p < 0.01$ ).

**Conclusions.** We conclude that bone marrow derived from femoral reaming could increase callus formation and alkaline phosphatase level significantly. It has positive effect in femoral fracture healing to increase osteoblast activity.

**Keywords:** medullary bone marrow, callus diameter, callus volume, fracture healing, alkaline phosphatase

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## Perbedaan Penyembuhan antara Fraktur Femur Tikus Putih yang Difiksasi dengan *Intramedullary Wire* Dengan dan Tanpa Pemberian Sumsum Tulang Medula

### ABSTRAK

**Pendahuluan.** Penanganan fraktur beserta penyembuhannya merupakan kesatuan proses yang banyak diteliti. Pemberian sumsum tulang autogen diketahui memberikan hasil yang baik dalam proses penyembuhan fraktur. Sumsum tulang mengandung sel osteoprogenitor yang membantu penyembuhan tulang. Tujuan penelitian ini adalah mengetahui penyembuhan pada patah tulang femur dengan pemberian sumsum tulang dari medula.

**Bahan dan cara kerja.** Dilakukan penanganan fraktur femur tikus putih *Sprague Dawley* dengan *intramedullary wire* dan pemberian sumsum tulang yang diperoleh dari hasil *reaming* medula femur. Efek pemberian tersebut terhadap healing fraktur dievaluasi dari pembentukan kalus (diameter, volume) dan kadar *alkaline phosphatase* dalam serum. Sejumlah 40 ekor tikus dibagi dalam 2 kelompok (kontrol dan perlakuan). Kelompok kontrol ditangani dengan *intramedullary wire* sedangkan kelompok perlakuan ditangani dengan *intramedullary wire* dengan ditambahkan sumsum tulang hasil reamer sejumlah 0,5 hingga 1 cc. Hewan coba diobservasi selama 30 hari, kemudian dievaluasi secara radiologis dan imunokimia melalui pengukuran kadar *bone alkaline phosphatase*.

**Hasil.** Dari hasil penelitian didapatkan diameter kalus kelompok perlakuan 34,1% lebih besar dari kelompok kontrol ( $p < 0,01$ ), volume kalus kelompok perlakuan 2,02 kali lebih besar dari kelompok kontrol ( $p < 0,01$ ), dan kadar *bone alkaline phosphatase* kelompok perlakuan 23,63% lebih besar dari kelompok kontrol ( $p < 0,01$ ).

**Simpulan.** Dari hasil penelitian diatas didapatkan bahwa pemberian sumsum tulang hasil *reaming* medula femur meningkatkan pembentukan kalus (diameter dan volume), serta meningkatkan kadar BAP seiring dengan peningkatan osteoblast pada proses penyembuhan fraktur.

**Kata kunci:** sumsum tulang, diameter kalus, volume kalus, penyembuhan fraktur, fosfatase alkali

### Introduction

Nowadays, treatment of fractures is experiencing rapid growth. Varieties of studies to accelerate process of fracture healing have been carried out. Time of union in the reamed nail group is faster because the reaming process produces autograft. Moreover, reaming process results in larger contact between nail and bone so that mechanical stability is increased.<sup>1,2</sup>

Hussain<sup>3</sup> demonstrated the advantages of intramedullary nailing, including less scar formation, shorter operation time, less blood loss, faster mobilization, and less incidence of joint stiffness.<sup>3</sup> Ways to accelerate bone healing treated with intramedullary nail have started to be studied.

Thanoon<sup>2</sup> did an experimental study in rabbits with femoral fracture which was treated with intramedullary nail and given an autogenous bone marrow taken from

the femoral head. Radiological callus on group which received bone marrow demonstrated significantly callus more than those which did not get a bone marrow.<sup>3</sup>

Hernigou<sup>4</sup> treated 43 patients with nonunion and bone defect using percutaneous bone graft, the healing time in group which are not given bone marrow is longer than the group which given marrow bone.

From those researches, it was found that addition of bone marrow accelerate the bone healing process. Bone marrow in those studies was obtained from iliac crest of pelvic bone in human. Bone marrow is also present in the medullary of long bone. Bone medulla is an area in any bone which holds bone marrow.<sup>3-7</sup>

To evaluate fracture healing process, measurement of callus from radiograph or direct measurement from fracture area is commonly utilized. In addition, several monitoring techniques of bone healing process such as

blood chemistry examination have been developed.<sup>8-10</sup>

Bone alkaline phosphatase (BAP) is one of chemistry examination that can be used for evaluating healing process. It is produced by osteoblasts, cells that produce matrix in fracture healing. BAP is a hydrolase enzyme to remove the phosphate from the alkaloid protein molecule. Increased levels of BAP show increased activity of osteoblasts.<sup>11-15</sup>

Al-Sobayil<sup>16</sup> conducted an experimental study using 20 male camels with mandibular fracture, evaluated serum bone alkaline phosphatase weekly, and found BAP to be a good biomarker of bone healing since the first week after surgery.

Based on those experiences, we conducted this study. Previous research has been done and found that bone marrow enhance healing process. Provision of bone marrow from other studies is usually taken from iliac crest and from head of the femur. Besides, the healing process of fractures fixed with intramedullary nailing with additional bone marrow and spinal fractures in the local area has never been investigated. The objectives of this study are to compare the diameter of callus and BAP between the fractures fixed with intramedullary nail with and without medullary marrow.

### Materials and methods

This study used Sprague-Dawley Rats aged 4-6 months, male, weighted 150 to 200 grams, and healthy (move actively, coat is not dull, has good response to stimuli). They were selected by simple random sampling technique and maintained in similar conditions and foods.

Subjects were divided into 2 group, namely study group and control group. Each group consists of 20 white rats. The control group was treated with intramedullary

wire while the study group was treated with medullary bone marrow in addition to intramedullary wire.

After intramuscular anesthesia using ketamine, the femurs were fractured with intramedullary K-wire inserted as technique described by Bonnarens and Einhorn. In treatment group, intramedullary bone marrow obtained by curettage and aspiration with 1 cc-syringe and added to the fracture site.

On the 30<sup>th</sup> day, callus diameter in mm was measured by digital calipers from the radiographs. Callus volume was determined by formula as introduced by Perkins and Skirving: callus volume =  $2\pi r_1(r_2-r_1)l$ .<sup>17</sup> The parameter for the formula was shown in figure 1.

BAP level was determined from analysis of blood using rat bone alkaline phosphatase reagent, BALP ELISA Kit, 96T and quantified using spectrophotometry. Results of measurement were then analyzed using Mann-Whitney test.

### Results

Mean diameter of callus was 4.17 mm in control group and 5.59 mm in treatment group. Callus in treatment group was 34.1% greater than in control group. Mean callus volume in control group was 41.23 mm<sup>3</sup> while in treatment group was 123.77 mm<sup>3</sup>. Callus volume in treatment group was 2.02 times greater than volume in control group.

Bone alkaline phosphatase in control group was 24.72 IU/L while in treatment group was 30.56 IU/L. Bone alkaline phosphatase in treatment group was 23.63% higher than in control group.

Mann-Whitney test revealed significant differences between control and treatment group regarding callus volume ( $p = 0.01$ ) and BAP level ( $p = 0.01$ ). They meant that addition of bone marrow increased callus volume and BAP level.

### Discussions

Bone marrow is well known to possess osteoprogenitor cells. In the case of fracture, administration of autologous bone marrow provides good results. Bone marrow has an important function in osteogenic. Osteogenic nature of bone marrow was first demonstrated by Geugeon in 1869.<sup>18-20</sup>

Our results showed increase in callus volume in treatment group. The finding is similar to a study by other authors, who also reported increased callus volume in rabbits' femoral fracture treated with autogenous bone marrow obtained from femoral head.<sup>4</sup>

In our study, BAP level was increased in treatment group. BAP is a biomarker of bone healing. It was

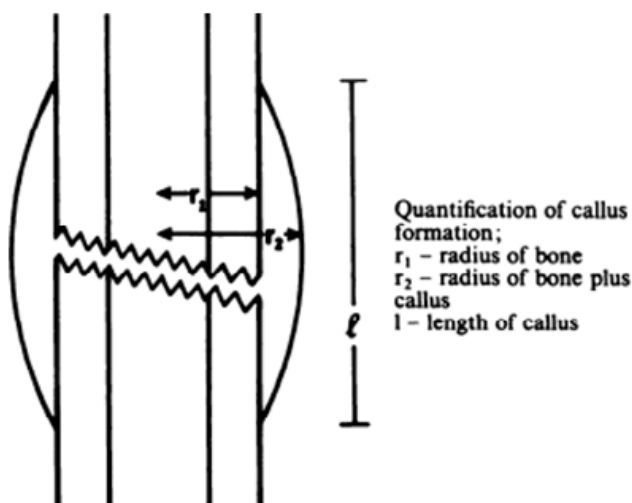


Figure 1. Measurement of callus volume

reported to be elevated in human long bone fractures due to trauma, and persisted until at least 1 year. BAP could serve as biomarker of bone formation even before radiological changes occurred.<sup>19,20</sup> Bone marrow contains osteoprogenitor cells that have properties to enhance formation of osteoblast. As receptors of BAP are found in osteoblasts, increased of osteoblast is marked by the increased of BAP. However, it should be noticed that using BAP as a marker of fracture healing is not without

limitation. BAP is not specific for bone healing, as it may increase in other circumstances such as in liver disease.

### Conclusions

We conclude that bone marrow derived from femoral reaming could increase callus formation and alkaline phosphatase level significantly. It has positive effect in femoral fracture healing to increase osteoblast activity.

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