# Open Osteotomy of ulna as a Model For Fracture Healing Studies in Rabbits

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**Summary:**

**Background:** Bone healing of fractures is still an open field for research due to its biological complexity. Adequately adapted experimental model is essential for understanding the factors influencing the biological process of bone healing. Rabbits provide a good animal model for bone healing studies; its bone had Haversian system similar to human.

**Material and Methods:** Six young locally breaded New Zeeland male rabbits were used in this study, the study conducted in animal house in college of medicine at November and December 2009. Surgical fracture (osteotomy) induced operatively in right ulna under general anesthesia by a hand saw. At the end of fifth weeks, the animals' scarified and the specimens taken for radiological, computerized tomography (CT) scan densimetry and histological examination carried out for bone healing in site of osteotomy

**Results**: All bone osteotomy united at end of fifth weeks macroscopically and radiologically. There was no significant difference in serum calcium, serum phosphate, and serum alkaline phosphatase preoperatively and at end of fifth weeks. The callus density was measured in site of osteotomy by CT scan densimetry and its mean was 302 ± 142. The histological examination of the bone at site of osteotomy show healing with woven bone predominantly with some lamellar bone and cartilage.

**Conclusion**: The present study demonstrated that the ulnar osteotomy without use of external splintage or internal fixation model is a new, easy to perform and can be used as a model for fractures healing studies.

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## Introduction:

Bone healing is complex event that involved the coordination of a variety of different process. Bone repair of fractures is still an open field for research due to its biological complexity1.Many experimental models describe in literature are useful for study fractures healing 2,3. The individual animals, based on their size and other characteristics, are used to show the effects of different interventions on bone healing 3. The models may have fractures created by closed or open means or osteotomies, and internal fixation, external fixation, or no fixation may be used 1, 2, 3 Fractures healing can be evaluated through clinical, radiological, mechanical, histological, chemical, or biological study2,3. Rabbits provide a good animal model for bone healing studies; its bone had Haversian system similar to human. Femoral, humeral, radial, ulnar, and tibial osteotomy used widely and usually used with internal fixation which have its effect one bone healing 2,3,4,5,6, 7, 7, 8. Open osteotomy had been described in fibula of immature rabbits without fixation or with internal fixation 2, 9, 10. The aim of this study was to present and to evaluate a model for fractures healing studies in experimental animal (rabbits) by open ulnar osteotomy without use of external splintage or internal fixation. Ulnar open osteotomy in young male rabbit without external splintage or internal fixation expected to be a new good model of bone healing.

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## Material and methods:

This study was approved by the research ethics committee at the College of Medicine, University of Mosul and follows the council for international organization of medical sciences ethical code for animal experimentation.

**Animals sample and Environment:** Six young male (4 months aged) locally breeded New Zeeland rabbits from animal house, College of Medicine, University of Mosul were used in this study at November and December 2009. Their average weight 1460 grams ranged between1250 grams and 1600grams. The animals were kept in separate metallic cages for one week for adaptation in animal's house in College of Medicine, Mosul University. In each cage one animal feed with standard ration and water

**Experimental technique:** Food was suspended eight to ten hours prior to administration of anesthesia. To decrease the vagal tonus, each animal received 0.2 mg/kg dose of atropine sulphate by intramuscular injection. Animals were anesthetized by intramuscular injection of ketamine (50 mg/kg of body weight) and intramuscular injection of diazepam (5.0 to 10.0mg/kg of body weight). Preoperative antimicrobial prophylaxes consisting of 50 mg/ kg of ceftrioxone were injected subcutaneously in proximal part of the same limb.

Sample of blood aspirated to measure serum calcium, phosphate, and alkaline phosphatase. The right forelimb were shaved and cleaned by betadine solution. Under an aseptic conditions technique, the Right ulna of each animal was accessed by an anteromedial longitudinal skin incision of approximately 20 mm. After division of the skin and subcutaneous tissue, the fascia, the muscles and tendon were retracted and the periosteum were opened and dissected from the ulna. The ulna shaft was exposed; osteotomy was performed on the exposed portion of the ulna by mean of a one mm blade thickness sterile hand saw. The incision was closed by layers, using absorbable 5-0 polyvycril sutures for the fascia and 4-0 monofilament PDS sutures for the skin, local dressing applied locally using sterile gauze covered with adhesive plaster. After five weeks sample of blood aspirated to measure serum calcium, phosphate, and alkaline phosphatase, animals were anesthetized again as described previously and terminated with a 2 ml intracardiac injection of potassium chloride. The right ulna of each animal was removed, after being dissected from the surrounding soft tissue.The samples examined radiologically by Siemen-Sirography fluoroscopy equipment 62 K.T.; the KV used in taking x-ray is 30 KV, 50mA. The Computerized Tomography (CT ) scan examination carried out to measure the density of callus at the site of osteotomy .The CT scan equipment is light speed, multidetector equipment, General Electric (GE), 32 Yokogawa Medical System, taken TA 0.6 mm slice thickness. The mean of five points taken at the site of osteotomy to measure the density of callus and five points at the normal bone proximal to osteotomy, the means and standard deviations of these values calculated. The sites of osteotomy were carefully exposed by removal of all the soft tissue. The ulnar bones were removed, and fixed with 10% formaldehyde solution. After fixation, they were decalcified in 10% foramic acid. The decalcification process demineralized the bone, leaving only the soft tissues and bone matrix. This was done to ensure that thin sections cold be examined histological. Thin sections embedded in paraffin wax were cut and stained with haematoxylin and eosin.

**Statistical analysis:** Results are reported as mean ± standard deviation. The student (t) test used to calculate the differences between two means. The p value was considered a significant if it is less than 0.05.

**Results:**

All animals survived to the end the study. Neither wound infection nor wound dehiscence were observed in the entire animals group. All animal had normal serum calcium (3.3 ± 0.2 mmol/dl), serum phosphate (1.32± 0.16 mmol/dl), and serum alkaline phosphatase (11.8 ± 2.8 IU unit/ dl) at time of osteotomy. Five week after osteotomy, there was no statistically significant difference ($p > 0.05$) in the means of serum calcium (3.2 ± 0.16 mmol/dl), serum phosphate (1.35 ± 0.18 mmol/dl), and serum alkaline phosphatase (12.2 ± 4.6 IU unit/ dl)' (table: 1). Macroscopic examination demonstrates that all osteotomies were united by the end of the study. All bone was united radiologically after 5 weeks, (fig: 1). The mean of CT scan density of callus at the site of osteotomy was 302 with a standard deviation142. The mean of CT scan density in normal bone proximal to site of osteotomy was 947 with a standarddeviation219, (fig-2). The Histopathological examinations of the osteotomy site shows healed bone with predominantly woven bone with area of mature (lamellar) bone with some area of cartilage, there is no evidence of infection or foreign body reaction ,( fig-3).

**Fig: 1 – Radiological examination (X-ray) of forearm shows healed osteotomy in ulna of rabbit in stage of union.**

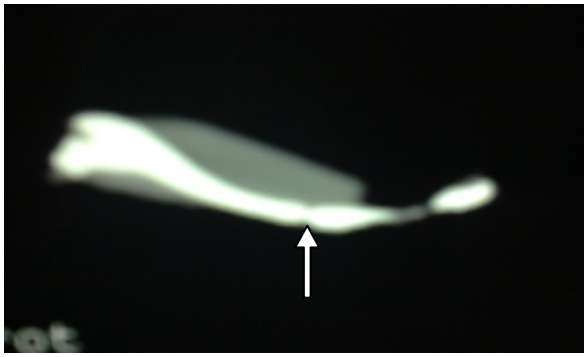

**Fig: 2- CT scan of the ulna with healed site of osteotomy, the density of callus at the site of osteotomy was measured to assess the bone healing**.

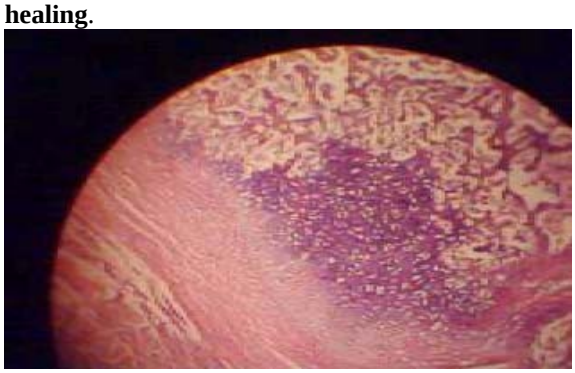

**fig. 3: Histological examination show new bone formation at site of osteotomy in different stages of healing (woven bone with lamellar bone and some cartilage areas) without any evidence of chronic inflammation or giant cell reaction at osteotomy site.**

**Table: 1- The serum calcium, phosphate, alkaline phosphatase of rabbits in preoperative and at end of fifth weeks.**

| Samples | Preoperative values | | Values at end of fifth weeks | | P value | Significance |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | | |
| Serum calcium | 3.3 | 0.2 | 3.2 | 0.16 | > 0.05 | Not significant |
| Serum phosphate | 1.32 | 0.16 | 1.35 | 0.18 | > 0.05 | Not significant |
| Serum alkaline phosphatase | 11.8 | 2.8 | 12.2 | 4.6 | > 0.05 | Not significant |

**Discussion:**

Adequately adapted experimental model is essential for understanding the factors influencing the biological process of bone healing. Many experimental models were used to study healing 2,3. These models not always give adequate parameters for human, Every model has its advantages and disadvantages 2,3. The difficulties in gathering data from studied models come from the need of using fixation devices which affect bone healing, excessive mobility of fracture when not fixed, animal size and its care, and difficulties in obtaining uniform fractures when manually produced 2,3,4,5. The rabbits was chosen as the animal model because it is widely used in studies of bone preparations 2,3,4, 5, 6, 7, 8. Their size is small enough to allow the use of moderately large number of animals, but is large enough to operate on it, fixation of fractures, drug administration and biophysical study [4, 5, 6, 7, 8]. Rabbit had Haversian bone system similar to humans with similar bone healing mechanism 2, 3, 9, 10. It is estimated that five weeks is enough for bone healing and all animals show good callus in radiographic examination with good bone density in CT densimetry examination, and the histological demonstrated that healing process was advanced. Different models of experimental osteotomy used in the rabbits. Femoral, humeral, tibial, and radial osteotomies usually need internal or external fixations which have many effects on natural bone healing 2, 3,4,5,6, 7. The fibula used as a model of osteotomy in rabbits without external or internal fixation 2, 9, 10, but this bone is small to operate and harvested. Rabbits ulnar osteotomy used with internal fixation which have its effects on bone healing and cause more damage to the local tissues 8. The ulna selected for open osteotomy without fixation because it is easy to access and had good size and it is easy to harvest. The radius and ulna, though firmly attached together, are not ankylosed 11. This made ulnar osteotomy stable and need no internal or external fixation, to avoid the effect of fixation on bone healing. The osteotomy done by hand saw to avoid local heat production induce by high velocity osteotomy devices, to minimize the local tissue damage, and to be nearer to natural fractures. The small number of animals used in this experiment is to avoid unnecessary losses in animals and due to shortage of facilities, but we think that it is sufficient to get a conclusion and stimulate more wide clinical studies when financial and technical support is available. The small number of animals used in this experiment is also fit with animal studies protocol which insist on the principle, that the animal experiments should be designed to minimize the number of animal used 12. The serum calcium, serum phosphate, serum alkaline phosphatase in normal rabbits was 3.2-3.7mmol/L, 1.28- 1.92 mmol/l, and 10-70 IU/L respectively 11. The results in experiment were normal preoperatively and five week after experiment, and there was no significant difference between the preoperative results and the results after five weeks before animals killing, (table: 1). Small animal (rat and mouse) is easy to handle but the use of standard surgical osteotomy is difficult; adding fixation device to stabilize the bone influence bone healing is also difficult, and not suitable for biomaterial and biomechanical tests 2, 13, 14. Rat and mouse had different histology of bone (no Haversian system) and had different bone healing from human 2, 13, 14. Large animals such as dog, sheep, goat, horses and others are expensive, difficult to handle, need complicated care and internal fixation and its care is very difficult for a regular investigators 2, 15, 16, 17. The use of young animal with early mature skeleton makes the model similar to young adult and adolescent populations 2,3 . Biomechanical studies were not performed on ulnar bone in this study because of shortage of facilities, but we think that mechanical studies could be done easily on this model when it is available. Histomorphometric evaluation of the bone could be performed in this model when the facilities become available. We conclude that the ulnar osteotomy in young male rabbit presented in this report is a new, easy to performed and practical model for studies of bone healing without external splintage or internal fixation.

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