

# Correlation between Computerized Tomography Densitometry and Histomorphometry in Assessment of Bone Healing

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## Summary:

**Background:** Assessment of fracture healing is a common problem in orthopedic practice and research. Computerized Tomography (CT) is a reliable tool for quantification of the fracture repair process in experimental animals. Histomorphometric evaluation provides a clear quantitative evidence of the bone healing process. The evaluation by micro-architectures in healing bone showed variable correlation between CT and histomorphometry.

**Material and methods:** open ulnar osteotomy induced in twenty young male rabbits under general anesthesia without internal fixation, and divided into five groups. A group of animals were sacrificed at end of 2<sup>nd</sup> week, 3<sup>rd</sup> week, 4<sup>th</sup> week, 5<sup>th</sup> week, and 6<sup>th</sup> week. The right ulna isolated and the bone specimens taken for radiological examination, CT scan densitometry and histomorphometric evaluation carried out for the callus at the site of osteotomy for all animals in both group.

**Results:** The mean of histomorphometric evaluation of callus at the site of ulnar osteotomy at the end of second week were 2.9, and increased with duration to reach 9.3 at end of sixth week. The mean of CT densitometry of callus in site ulnar osteotomy at the end of second week were 96.2, and increased with duration to reach 723.3 at end of sixth week. The correlation coefficient between histomorphometric evaluations of callus in site ulnar osteotomy and value of CT densitometry measurements was 0.958, which considered very highly significant, (the P value < 0.0001).

**Conclusion:** The present study demonstrate that, there is strong correlation between CT scan bone densitometry of callus in site of osteotomy and the results of histomorphometric evaluation of callus. The CT scan bone densitometry of callus can be used to assess the bone healing in experimental and clinical studies, as it is noninvasive technique.

**Keywords:** Bone, Computerized Tomography, Densitometry, Histomorphometry, healing, rabbits.

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## Introduction

Bone healing is an extremely complex process which depends on the coordinated action of several cell lineages on a cascade of biological events and has always a major medical concern 1. Assessment of fracture healing is a common problem in orthopedic practice and research 2. Fractures healing can be evaluated through clinical, radiological, mechanical, histological, chemical, physical or biological studies 3.

Histomorphometry is the gold standard for assessing bone because it is the only method for direct in situ analysis of bone cells and their activities 4, 5. Histomorphometric (quantitative) evaluation of the callus shown to be compatible with bone healing achieved in qualitative experimental methods 6. Advent of x-ray computed tomography (CT) has provided the opportunity to quantitatively and non-destructively assess bone structure and density 7. Bone mineral density measurement by CT is noninvasive, and a reliable tool for quantification of

the fracture repair process in experimental animals 8. The mineral density of callus correlated positively with callus strength and stiffness 7. By reviewing the available literatures, there were no registered studies on the correlation between CT densitometry and the histomorphometric evaluation of bone healing in osteotomy of long bone in rabbits. The aim of this study is to evaluate the correlation between CT callus densitometry and callus histomorphometry in assessment of bone healing at site osteotomy of ulna in experimental animals (rabbits).

## Material and methods:

This study was approved by the scientific research 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:** Twenty young male (4 months aged) locally bred New Zealand rabbits from animal house, College of Medicine, University of Mosul were used in this study at November 2009 to April 2010. Their average weight 1400 grams ranged between 1250 grams and 1600 grams. The animals were kept in separate metallic cages for one week for adaptation in

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animal's house. 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 ceftriaxone 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, no external splintage or surgical fixation were used.. The animals were kept in separate metallic cages, feed with standard ration and water.

The animals divided into five groups (four in each), the first animals' group sacrificed at end of 2<sup>nd</sup> week, second group sacrificed at end of 3<sup>d</sup> week, third group at end of 4<sup>th</sup> week, forth group at end of 5<sup>th</sup> week, and fifth group at end of 6<sup>th</sup> week. Animals were anesthetized as described previously, a sample of blood aspirated to measure serum calcium, phosphate, and alkaline phosphatase, and killed with a 2 ml intracardiac injection of potassium chloride. The right ulna of each animal was removed, 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 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. The site of osteotomy examined histologically. The progression of fracture-healing in each specimen was quantified with the use of a scale that assigns a grade based on the relative percentages of fibrous tissue, cartilage, woven bone, and mature bone in the callus (histomorphometric evaluation )<sup>8</sup>. Grade 1 indicates fibrous tissue; grade 2, predominantly fibrous tissue with some cartilage; grade 3, equal amounts of fibrous tissue and cartilage; grade 4, all cartilage; grade 5, predominantly cartilage with some woven bone; grade 6, equal amounts of cartilage and woven bone; grade 7, predominantly woven bone with some cartilage; grade 8, entirely woven bone; grade 9, woven bone and some mature bone; and grade 10, lamellar (mature) bone. The histomorphometric examination carried out in Al-Jumhori hospital laboratory. The grading was done blindly without knowing which treatment had been given. The mean of fracture healing scores were calculated for each group.

**Statistical analysis:** Results are reported as mean  $\pm$  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. The correlation tested by Pearson correlation coefficient. Statistical analysis was conducted using the statistical package for social sciences for window version 11(SPSS Inc., Chicago. Illinois.).

### Results:

All animals survived to the end the study. Neither wound infection nor wound dehiscence were observed in the animals of either group. Macroscopic evaluations demonstrate that all osteotomies were united by the end of 4<sup>th</sup> week in the study. Radiological examination shows that osteotomies were at different stage of bone healing according to dating of scarification. All animal at time of osteotomy had normal serum calcium (the mean was  $3.4 \pm 0.22$  mmol/dl), serum phosphate (the mean was  $1.45 \pm 0.18$  mmol/dl), and serum alkaline phosphatase (the mean was  $11.6 \pm 2.4$  IU unit/ dl) 9. At the time of scarifying animal, the serum calcium was normal (the mean was  $3.4 \pm 0.14$  mmol/dl), the serum phosphate was normal (the mean was  $1.42 \pm 0.1$  mmol/dl), and serum alkaline phosphatase was normal (the mean was  $12. \pm 1.6$  IU unit/ dl) 9. By using student (t) test to compare the differences between two mean, there was no statistically significant difference ( $p > 0.05$ ) in the means of serum calcium, phosphate, alkaline phosphatase at starting experiment and at scarifying animals.

The mean of histomorphometric evaluations of callus in site ulnar osteotomy at the end of second week were 2.9, and increased with duration to reach 4.3, 5.8, 7.95, 9.3 at end of 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> week

respectively, while in normal bone proximal to osteotomy is 10 (Table-1). The mean of CT densimetry measurements of callus in site ulnar osteotomy at the end of second week were 96.2, and increased with duration to reach 222.3, 321, 578.3, 723.3 at end of 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> week respectively, while in normal bone proximal to osteotomy 954 (Table-1).

The result of statistical analysis showed a Pearson correlation coefficient of 0.958 between histomorphometric evaluations of callus in site ulnar osteotomy and value of CT densimetry measurements of callus in site ulnar osteotomy, the P value < 0.0001 which was considered very highly significant.

**Table-1: The mean of Histomorphometric evaluation and CT scan densimetry at site of osteotomy according to duration of bone healing.**

Groups	Histomorphometric evaluation (mean ± SD)	CT scan densimetry (mean ± SD)
2 <sup>nd</sup> weeks	2.9 ± 0.79	96.2 ± 32.9
3 <sup>rd</sup> weeks	4.3 ± 0.98	222.5 ± 79.5
4 <sup>th</sup> weeks	5.8 ± 1.2	321 ± 85.3
5 <sup>th</sup> weeks	7.95 ± 0.83	578.3 ± 133.6
6 <sup>th</sup> weeks	9.3 ± 0.66	723.3 ± 154.5
Normal bone proximal to osteotomy site.	10	954 ± 186.5

**Discussion:**

The rabbits were chosen as the animal model because it is widely used in studies of bone preparations and its bone similar to human bone<sup>9</sup>. The ulna selected because it is easy to access, had good size, its fixation not essential because of close relation with radius to get rid of the effect of the fixation, and it is easy to harvest<sup>9</sup>. The number of animals used in this experiment is sufficient to get a conclusion and to stimulate more wide clinical studies, this also fit with statement of American Academy of Orthopedic Surgeon in animal use in experiment, that the protocol should be designed to minimize the number of animal used<sup>10</sup>. The animals were (normocalcaemic and normophosphataemic) at time of osteotomy and at end of study. The normal serum calcium, serum phosphate, serum alkaline phosphatase in normal rabbit was 3.0- 4.2 mmol/l, 1.28- 1.92 mmol/l, and 10-70 IU/L respectively<sup>9</sup>. This finding indicates that animals are healthy and had no systemic bone disease through all the time of study.

In our study, the correlation between histomorphometric evaluations of callus in site ulnar osteotomy and value of CT densimetry measurements was statistically very highly significant. This indicate that the CT densimetry, can be used to assess the stage of bone healing in experimental studies and can be apply clinically to assess the level of union after fracture and osteotomies. Bone mineral density measurement by Computerized Tomography (CT) is noninvasive,

nondestructive and a reliable tool for quantification of the fracture repair process in experimental animals<sup>2</sup>. The mineral density of callus correlated positively with callus strength and stiffness<sup>7, 11</sup>.

Bone histomorphometry is an important research tool in the field of bone metabolism and provides information that is not available by any other investigative approach<sup>12</sup>. Bone histomorphometry is a quantitative histological examination of decalcified and undecalcified bone biopsy performed to obtain quantitative information on bone remodeling and structure<sup>12, 13</sup>. Histomorphometric evaluation provides clear evidence of the healing process<sup>14</sup>. Cui et al proved that bone histomorphometry is useful for predicting early physiological and pathological changes in ovariectomized rats<sup>15</sup>.

The regional cortical bone mineral density measured by CT is useful to assess changes in material properties of bone associated with the degrees of mineralization<sup>16</sup>. Three dimension micro-CT measurements are comparable to those of standard histomorphometry in assessment of transmenopausal trabecular bone structure<sup>17</sup>. The evaluation by micro-architectures in regenerated bone showed variable correlation between micro-computed tomography and histomorphometry<sup>14</sup>. Coret et al found in cadaveric calcaneus that, trabecular bone structure measures determined on CT image show highly significant correlation with those determined using histomorphometry<sup>18</sup>. The level of correlation varies according to the type of method used for characterizing bone structure, however, and the strongest correlation was found for the most basic features<sup>18</sup>. Borah et al showed that there is significant correlation between micro-computed tomography results and histomorphometry indices of turnover in transiliac biopsies<sup>19</sup>. Xing et al confirmed that, the geometric densimetric and mechanical properties in cortical and trabecular bones of rat can be well described by peripheral quantitative CT<sup>20</sup>.

The dual energy x-ray absorptiometry (DEXA) in our work in measurement of bone density in site of osteotomy. Libouban et al found that DEXA over estimated bone mineral density of cortical by 11%, and the over estimation was found to be clearly depend on the net mineral content of the bone<sup>21</sup>. Folekemeier et al found that stiffness measurement are a better quantitative indicator of bone healing than bone mineral density and content measurement by DEXA<sup>22</sup>. The dual energy x-ray absorptiometry is less efficient than histological histomorphometric method of diagnosis with low specificity, and there was moderate correlation between DEXA and histomorphometry in assessment and diagnosis of osteoporosis in hip fractures patients<sup>5</sup>. Wu in his assessment of bone mineral density in postmenopausal women with or without vertebral fractures using CT scan and DEXA found that CT scan was better in the assessment<sup>23</sup>.

In conclusion, our study demonstrates that there is strong correlation between CT scan bone densimetry of callus in site of osteotomy and the results of histomorphometric evaluation of callus, the CT scan bone densimetry can be used in evaluation of bone healing in experimental and clinical studies as noninvasive technique.

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