Histopathological evaluation of the platelets rich fibrin and bone marrow on healing of experimental induced distal radial fracture in local dogs

[1]Thanoon M.G., [2] M. J. Eesa and [3]Alkenanny E.R.

[1]Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq. [2]Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq. [3]Department of Pathology, College of Dentistry, University of Al Iraqia, Baghdad, Iraq.

E-mail: thanoonmoyaser@yahoo.com

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Summary

The aim of this study was to evaluate the effects of platelets rich fibrin and bone marrow on healing of distal radial fracture in local dogs. Twenty four adult animals (males and non-pregnant females of local breed dogs); had mean age 2.6 ±0.15 years and body weight 24.58 ±1.07 kgs were used. The experimental animals were randomly divided into three equal groups. First group (Control group), transverse fracture was induced at the distal portion of radial bone and immobilized by using Plaster of Paris, the fracture line didn't treated with any substance. Second group (Platelets Rich Fibrin group), in which the fracture line was surrounded by the platelets rich fibrin. Third group (Bone Marrow group) in which the fracture line was surrounded by autologous bone marrow. The histopathological results confirmed that the third group was the best one in its response for fractured bone healing in both periods sixth and tenth week, while the second group was came in the second rank, whereas the first group was the slowest response for fractured bone healing, represented by trabecular bone formation. The concentration rates of calcium and alkaline phosphatase enzyme increased at the weeks that follow surgical operation. In conclusion, the using of bone marrow and platelets rich fibrin are enhance the healing of distal radial fracture.

Keywords: Fracture, Radial bone, Bone marrow, Platelets rich fibrin.

Introduction

The distal radial fracture is representing 17.8 % of all canine fractures and about 85% of the all radius fractures. The traumatic injuries which result from traffic accidents, falling, fighting, and gunshot are the main causes of distal radial fractures (1). The distal radial region suffers from low blood supply rather than little soft tissue which may contribute to the delay or incomplete healing of their fractures. The delay-union, mal-union or even non-union healing is common in distal radial fracture especially in toy breeds dogs (2). The platelets rich fibrin (PRF) is a new therapeutic concept of the second generation of platelets concentrate in a fibrin membrane. The platelets regulate the healing process including cellular migration, proliferation, and angiogenesis; it also controls the cell apoptosis and interaction with progenitor cells. Therefore the PRF is considered a bioactive surgical additive material having crucial role not only as hemostasis but also regulate inflammatory process with accelerate wound healing and bone regeneration (3). Platelet is highly specialized secretory cell releases growth factors with cytokines to improve healing process. These platelet growth factors enhance chemotaxis, induction, differentiation and proliferation of stem cells, progenitor osteoblasts, and influences on bone cells activity. The effective role of platelets is to reduce the excessive inflammation as well as acting as a scaffold to bridge the fracture gap and for the rapid and beneficial transferring of mesenchymal stem cells, progenitor cells, and growth factors(4).

Bone marrow (BM) is a soft gelatinous spongy tissue found in the cavities of body bones, also known as the medulla ossium (5). Bone marrow contains two types of stem cells: Hematopoietic stem cells (HSC), which produce different types of blood cells, and Mesenchymal stem cells (MSC), which can

produce various cells of body tissues, including myocytes, chondrocytes, osteoblasts, adipocytes and endothelial stem cells that form blood vessels (6). The autologous bone marrow transplantation is one of the most advantageous cell sources for promoting both osteogenesis and angiogenesis (7). The aim of this study was evaluated of platelets rich fibrin and bone marrow on healing of distal radial fracture in local dogs.

Materials and Methods

Twenty four adult animals (males and non-pregnant females of stray local breed dogs) were used in this study, mean aged 2.6 ±0.15 years, and the mean body weight 24.58 ±1.07 kgs. The animals housed indoor under the same feed and management conditions, in the animals' house of College of Veterinary Medicine, University of Baghdad, after obtaining an official approval from the ethical committee of the college. The experimental animals were randomly divided into three equal groups. The first group (Control group), the second group (Platelets Rich Fibrin group), and the third group (Bone Marrow group)

The surgical operation was conducted under strict aseptic condition preparation and condition. Food was withheld for 12 hours and water for two hours before operation. In the first group, transverse fracture was induced at the distal portion of radial bone in all experimental animals, A protocol of general anesthesia include a mixture of Xylazine hydrochloride 2% with Ketamine hydrochloride 10% at a dose 5 mg/kg and 15 mg/kg B.W., intramuscular respectively. The cranio-medial aspect is the best surgical approach for inducing distal radial fracture by using a wire saw (8). The fractured bone immobilized by Plaster of Paris. Postoperative care including daily wound management and dressing, with intramuscularly injection of penicillin-streptomycin at a dose of 10000 IU, 20 mg/kg B.W., respectively for 5 consecutive days. The stitches were removed at 10-12 days after operation. All experimental animals were clinically observed daily for 2 weeks then weekly until the end of operation (the follow up10 weeks). In the second group similar to the first group, but platelets rich fibrin was prepared according to (9) and was covered the fracture line. While in the third group, autologous bone marrow was aspirated from the proximal end of femoral bone then injected at the fracture line.

Bone biopsy was taken from all experimental animals in two periods at 6th and 10th weeks after inducing distal radial fracture. The bone biopsy was directly kept in the 10% of neutral formalin for 72 hours, then decalcification by passing it in Formic acid with Sodium citrate solution as stated in (10). Then these biopsies were submitted for a series of passes by Alcohol, Xylol and paraffin wax until they were ready for the histopathological sections. The tissue slices were dissected into thickness didn't exceed 5µ. These histological slices were stained by two different dyes; Hematoxylin and Eosin (H&E), and Masson Trichrom. The tissue slices were examined under the light microscope for detecting the differences of histopathological changes between the experimental groups; and for identifying the best group in their response to fracture bone healing .

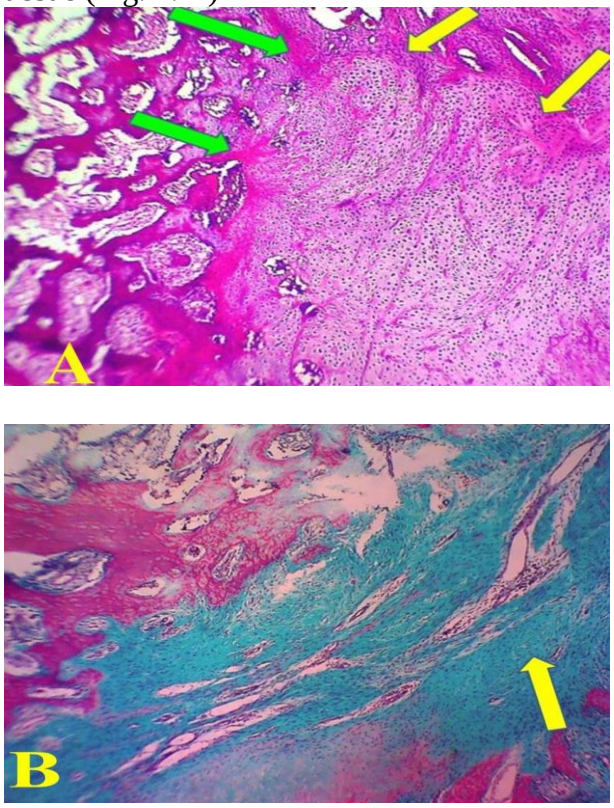
The total calcium and alkaline phosphatase enzyme concentration were measured by colorimetric method using a "Spinreact" kit for calcium, and "Biolabo" kit for alkaline phosphatase enzyme. Blood samples were drawn at the zero time before the operation, then at 2, 4, and 6 weeks after operation, from all experimental animals. Data submitted to statistical analysis using (ANOVA) and (LSD) used to differentiate among the means of parameters by using Sigma Stat (Jandel scientific software V3.1).

Results and Discussion

The surgical site of all experimental animals showed slight swelling without any inflammatory or serous secretions. Most inflammatory signs appeared as a simple swelling and redness at the area of operation disappeared within 3-5 days after operation. The lameness was evidence in all experimental animals. The third group showed their ability to use the broken limb at the end of the second week post operation, while the animals of second group showed their ability to bearing its weight on the fractured limb at the third week after operation, whereas animals of the first group were late in using the broken limb compared to the second and third groups.

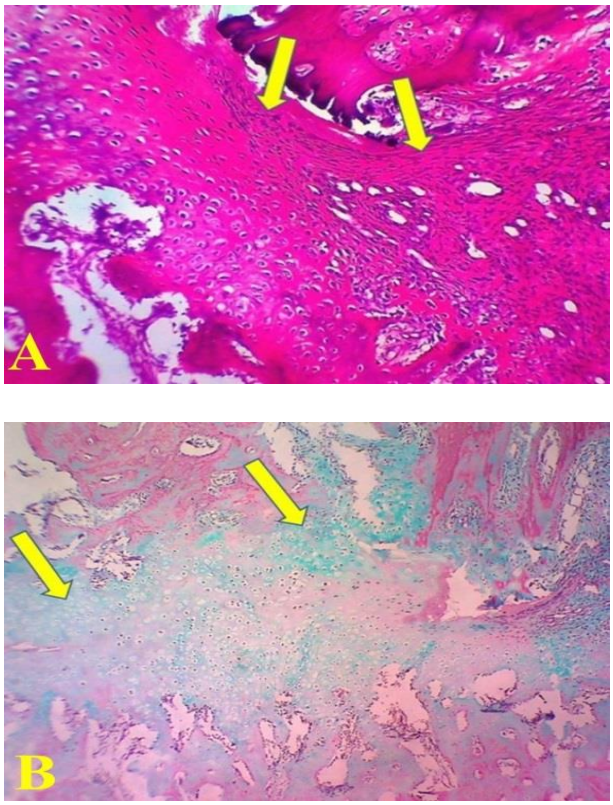
These results coincides by other authors (11 and 12), whom said that, the lameness is clinically cleared when the distal radial fracture accompanied by ulnar fracture and is less when only distal radial fracture without ulnar fracture.

The histopathological examination at the 6th week after operation: In the first group, showed a proliferation of fibrous tissue and atrophy in the cartilaginous tissue with absence of new bony tissue, and the gap that between the ends of fractured bone filled with fibrous tissue with few of fibrocartilaginous tissue (Fig. 1: A), and with the Masson Trichrom stain confirm presence of fibrous tissue with few amounts of fibrocartilaginous tissue (Fig. 1: B).



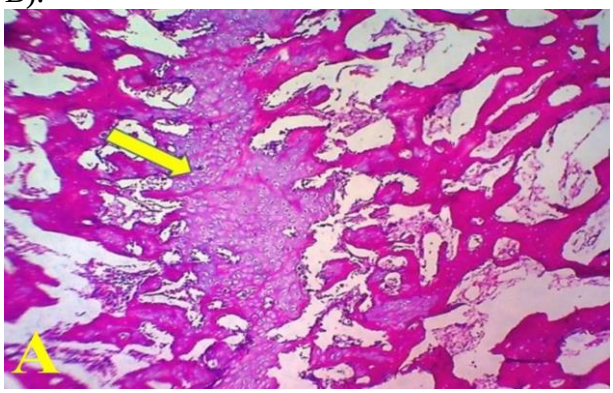
Figure, 1: Histopathological section of radial bone after inducing distal radial fracture, in the first group at the sixth week, shown fibrous tissue (yellow arrow) in the fracture gap, with fibrocartilaginous tissue (green arrow). A: H&E X40, B: Masson trichrom X40.

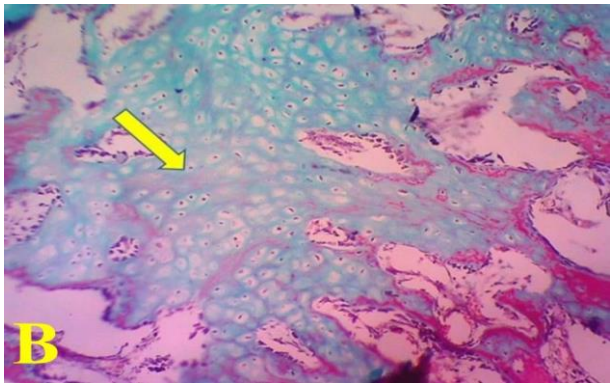
In the second group there was a proliferation and density of fibrous tissue in larger quantities of fibrocartilaginous tissue. The gap between the two ends of the fractured bone was filled by fibrous tissue as well as fibrocartilaginous tissue (Fig. 2: A and B).



Figure, 2: In the second group at the sixth week, shown increase of proliferation and density of fibrous tissue, as well as fibrocartilaginous tissue in the fractured bone gap (yellow arrow). A: H&E X40, B: Masson Trichrom X40.

In the third group was observed that the fibrocartilaginous tissue as a prevalent in the fractured bone gap with a few fibrous tissue, and in some sections there is ossification with transformation of cartilaginous tissue to the osseous tissue (woven bone), (Fig. 3: A and B).





Figure, 3: In the third group at the sixth week, shown presence of fibrocartilaginous tissue with few amounts of fibrous tissue (arrow) in the fractured bone gap. A: H&E X40, B: Masson trichrom X40.

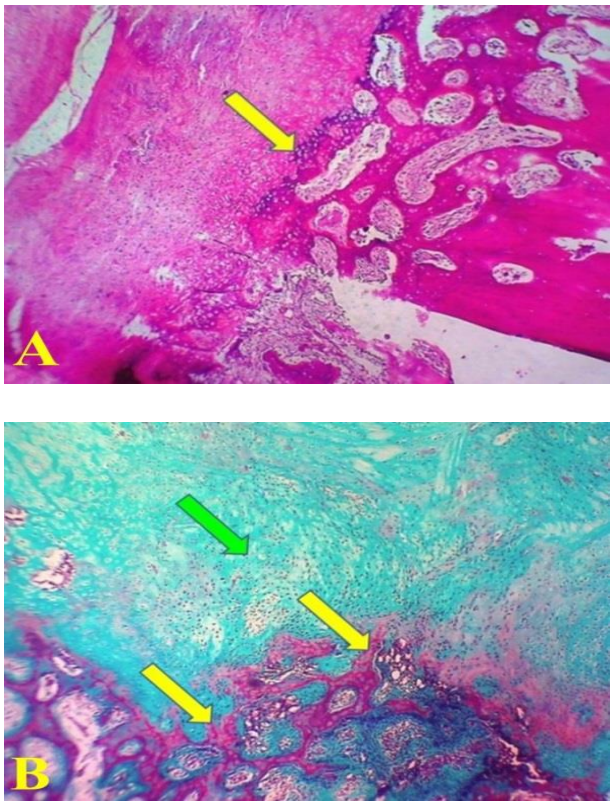
In the third group, observed that the fibrocartilaginous tissue was prevalent in the fractured bone gap with a few of fibrous tissue, and in some tissue sections, there is deposition of calcium with transformation of cartilaginous tissue to the woven bone type; this is may be due to presence of local stem cells as well as mesenchymal stem cells which presents in implanted bone marrow, which have ability to multiply and divided into different specialized body cells especially chondroblast, osteoblast, and this agree with other researchers (6 and 13). Who confirmed that the mesenchymal stem cells having an ability to multiply and differentiation into several types of body cells, including chondroblasts, osteoblasts and osteocytes, this is observed in presence of abundance of fibrocartilaginous and cartilaginous in many tissue sections as well as few of bone trabeculae.

In the second group was observed presence of fibrocartilaginous tissue in larger quantities than fibrous tissue, and the fracture gap was filled by the fibrocartilaginous tissue rather than fibrous tissue. This is may be due to presence of platelets rich fibrin which having a concentrated platelets, and this platelets having an ability to stimulate stem cells for division, migration, multiplication and differentiation in to different body cells that needed, especially chondroblasts and osteoblasts, this is confirmed by many workers (14 and 15). Who said that platelets contains many bioactive substances, including various growth factors, which are attributed in regulatory role of cells migration, proliferation, differentiation and maturity of various cells that have a large role in inflammatory and healing processes, as well as its role in the production of extracellular matrix; therefore the platelets having a significant benefit effects in bone healing.

In the first group noticed a great proliferation of fibrous tissue with a little of fibrocartilaginous tissue, and without formation of new bone tissue in the fracture gap. The first group has been excluded from the addition of stem cells that presents in the bone marrow as well as from the bioactive substances; especially growth factors which presents in the platelets and therefore observed a lack in formation of fibrocartilaginous and cartilaginous tissues compared with the second and third groups. The presence of fibrous tissue in abundance, which will later transformed in to fibrocartilaginous tissue, and this is coincide with many researchers (16 and 17). Who confirmed that the macrophage cells are acting as scavenger cells to remove the blood clots and dead cells by process of phagocytosis, while the osteoclasts cells works to remove cellular bone debris and dead ends of fractured bone, where the macrophages cells secrete their inflammatory and chemical substances, which begins to stimulate with activation of fibroblasts cells, mesenchymal stem cells and osteoblast that presents in the local area of bone marrow, periosteum and endothelium of capillary vessels.

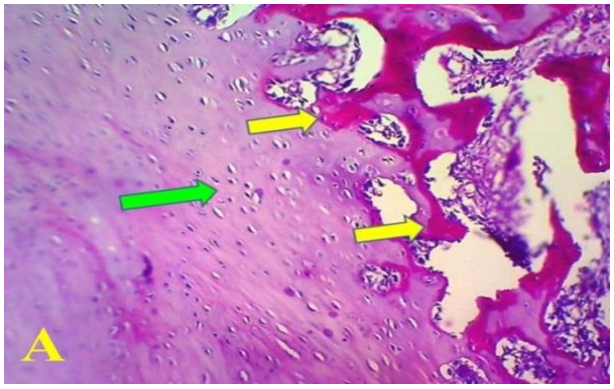
The results of examination at the 10th week after operation: In the first group, histopathological showed a proliferation of fibrous tissue as well as a proliferation of fibrocartilaginous tissue. The two ends of the

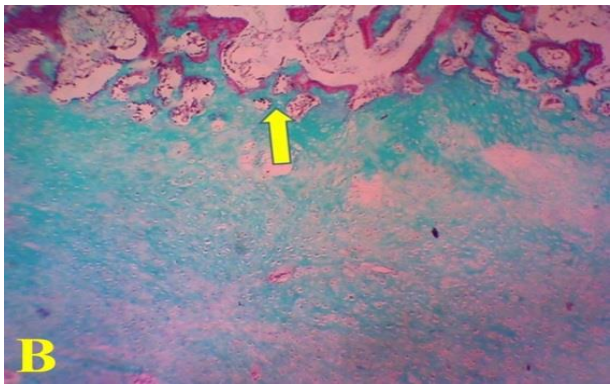
fractured bone have been linked by fibrocartilaginous tissue with few ossifications of some cartilage cells, (Fig. 4: A and B).



Figure, 4: In the first group at the tenth week, shown proliferation of fibrocartilaginous tissue in quantities exceeding the fibrous tissue (green arrow) with ossification of some chondrocytes (yellow arrow). A: H&E X40, B: Masson trichrom X40

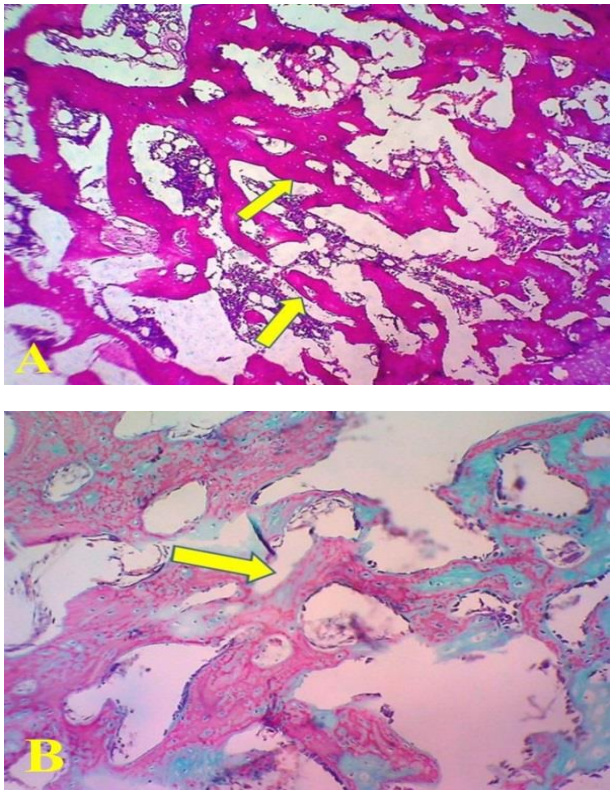
In the second group, the histopathological examination showed a significant proliferation of fibrocartilaginous tissue with ossifications of some chondrocytes rather than formation of new trabecular bone in many fractured areas, (Fig. 5: A and B).





Figure, 5: In the second group at the tenth week, shown a significant proliferation of fibrocartilaginous tissue (green arrow) with ossifications of chondrocytes (yellow arrow) as well as new formation of bone trabeculae. A: H&E X40, B: Masson trichrom X40.

In the third group noticed proliferation of cartilaginous tissue and transformed into a new boney tissue, with presence of many new bone trabeculae, (Fig. 6 A and B).



Figure, 6: In the third group at the tenth week, shown proliferation of cartilaginous tissue and its transformation into a new boney tissue, with presence of many new bone trabeculae (arrow). A: H&E X40, B: Masson trichrom X40.

This is due to the division, proliferation and differentiation of stem cells into osteoblasts and osteocytes cells in large quantities; this is confirmed with many researchers (17 and 18). Who said that the newly woven bone replaced of soft callus and this represents the early stages of calcium deposition in fibrocartilaginous scaffold and eventually leaves behind a partially calcified cartilage matrix with deposition of woven bone on the cartilaginous scaffold.

In the second group, the histopathological examination revealed a significant proliferation of fibrocartilaginous tissue with calcification of cartilaginous tissue as well as formation of new trabeculae bone in many fractured areas. The positive effective role of adding platelets rich fibrin on the fractured site is to restrict excessive inflammation rather than act as a scaffold to bridge the fractured gap for rapid passing and transferring of stem cells, progenitor cells as well as growth factors for rapid access to the fracture healing stage. This may be attributed to the presence of cytokines agents, growth factors of localized platelets and those implanted by platelets rich fibrin. Which activate the chemical attraction and differentiation of progenitor's cells, and tissue regeneration by stimulating the formation of new blood vessels to bring nutrients, stem cells and progenitor's cells to the site of injury and stimulate healing; this is agreements with other researchers (19 and 20). Who confirmed that platelets are the natural source of growth factors, cytokines, and other chemotaxis substances, which are attributed to the essential role in all stages of the healing process. Platelets have ability to modify and compensation of inflammation by increasing the inflammatory response for a short time with reducing the period of inflammation to promote healing stage.

In the first group, the histopathological examination revealed that the proliferation of fibrous tissue as well as the proliferation of fibrocartilaginous tissue in a quantity equivalent or superior to the fibrous tissue, the fractured ends were bridged by a fibrocartilaginous tissue with a slight calcification of some chondrocytes. This slowing down in fracture healing, lack of access to woven bone formation, and new bone trabeculae in the first group as its in the second and third groups, may be due to the absence of any bioactive material which having ability to speed up fracture healing such as bone marrow or concentrated platelets. Therefore, what reached the first group at the tenth week after operation is considered a normal condition, clinically acceptable and this confirmed by many researches (21 and 22). Who said that, the healing of the distal radial fractures takes longer time than rest of the body bones and may be the healing takes a period of 16 weeks. This is due to the lack of bloody supply of the distal third of the radial bone, as well as the lack of soft tissue that surrounds the distal portion of the radial bone.

These results indicated that the third group was superior in its response for fractured bone healing in both periods sixth and tenth week, while the second group was came in the second rank in its response for repairing of fractured bone, whereas the first group was the slowest response for fractured bone healing. The results of hematological changes exhibited an increasing in concentration rates of calcium and alkaline phosphatase enzyme at the weeks that follows surgical operation. This results with its statistical analysis summarized in the (table, 1) for calcium and (table, 2) for alkaline phosphatase enzyme.

Table, 1: Show calcium concentration rates with its statistical analysis in the three main groups of experiment.

Calcium concentration rates, mg/dL			
Times	First group	Second group	Third group
0	9.53 (±0.46) a	9.40 (±0.43) a	9.74 (±0.47) a
2 W	10.19 (±0.55) a	12.44 (±0.45) b	12.93 (±0.44) b
4 W	10.99 (±0.41) a	12.79 (±0.55) a, b	13.39 (±0.81) b
6 W	12.01(±0.88) a	10.86 (±0.48) a	11.00 (±0.44) a

*The symbol 0 represents the time before the operation, and 2W, 4W, 6W represents the time in weeks after the operation. The different small letters (a, b) indicate that there is a significant difference between the three groups at a probability P <0.05.

Table, 2: Show alkaline phosphatase enzyme concentration rates with its statistical analysis in the three main groups of experiment.

Mean of Alkaline Phosphatase concentration rates, U/dL			
Time	First group	Second group	Third group
0	20.29 (±0.87) a	21.10 (±1.55) a	18.80 (±1.66) a
2 W	22.39 (±1.70) a	28.38 (±1.60) b	35.01 (±2.38) c
4 W	26.31(±1.99) a	31.14 (±3.71) a, b	38.40 (±3.27) b
6 W	29.75 (±2.73) a	41.25 (±3.60) b	43.07 (±3.96) b

*The symbol 0 represents the time before the operation, and 2W, 4W, 6W represents the time in weeks after the operation. The different small letters (a, b) indicate that there is a significant difference between the three groups at a probability P <0.05.

The table, 1 shows; there is a significant difference at 2 week between the first group and rest groups, while at 4 week a significant difference between first and third group. In alkaline phosphatase (Table, 2) shows: there is a significant difference at 2 week, between first group with second and third groups, also between second and third groups. At 4 week there is a significant difference between first and third group. At 6 week there is a significant difference between the first group and rest groups.

The increasing of calcium concentrations in the weeks followed the operation refers to the effectiveness and activity of deposition of calcium and the formation of new trabeculae bone, and this is coincide by other authors (23 and 24), who said that, the calcium is necessary to maintain bone rigidity, and that it is necessary for the deposition of calcium in soft callus and repair of fractured bone.

The highest concentration rates of alkaline phosphatase enzyme were recorded at the sixth week. So the third group and the second group are the better fractured bone healing than the first group, depending on the serial rates of alkaline phosphatase enzyme concentrations in serum. This result is confirmed by many researchers (25 and 26); they prove that, the high levels of alkaline phosphatase in the serum indicated to the positive activity of osteoblast cells, and new bone formation. In conclusion, this experiment revealed that, the using of bone marrow and platelets rich fibrin were beneficial for improvement and enhanced healing of the distal radial fracture.

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التقييم النسجي المرضي لليفين الغني بالصفيحات الدموية ونخاع العظم على شفاء كسر الجزء القاصي لعظم الكعبرة في الكلاب المحلية

[1]ميسر غانم ذنون و [2]محمد جواد عيسى و [3]انتصار رحيم الكناني
[1] فرع الجراحة وتناسل الحيوان، كلية الطب البيطري، جامعة الموصل-العراق
[2]فرع الجراحة والتوليد، كلية الطب البيطري، جامعة بغداد-بغداد-العراق
[3]كلية طب الاسنان، الجامعة العراقية، بغداد-العراق
E-mail: thanoonmoyaser@yahoo.com

الخلاصة

ان الهدف من هذه الدراسة هو تقييم الليفين الغني بالصفيحات الدموية ونخاع العظم على شفاء الكسر الجزء القاصي لعظم الكعبرة في الكلاب المحلية. استخدم في هذه الدراسة 24 حيوانا ناضجا من الكلاب المحلية. معدل اعمارها 2.6 ±0.15 سنة ومعدل اوزانها 24.58 ±1.07 كغم، من الذكور والاناث غير الحوامل. قسمت حيوانات التجربة وبشكل عشوائي الى ثلاث مجاميع رئيسة متساوية. المجموعة الاولى (مجموعة السيطرة) احدث كسر مستعرض في الجزء القاصي لعظم الكعبرة وثبت بوساطة التثبيت الخارجي (الجبيرة)، ولم يعامل خط الكسر باي مادة. وفي المجموعة الثانية (مجموعة الليفين الغني بالصفيحات الدموية) احيط خط الكسر بالليفين الغني بالصفيحات الدموية، وفي المجموعه الثالثة (مجموعة الزرع الذاتي لنخاع العظم) تم حقن نخاع العظم والمسحوب من نفس الحيوان في مكان الكسر. أكدت النتائج النسجية المرضية أن المجموعة الثالثة كانت الأفضل في استجابتها لشفاء كسر الجزء القاصي لعظم الكعبرة وفي كِلا الفترتين الاسبوع السادسة والعاشرة، وجاءت المجموعة الثانية في المرتبة الثانية، في حين ان المجموعة الأولى كانت الأبطأ استجابة لشفاء كسر الجزء القاصي لعظم الكعبرة. ارتفعت معدلات تراكيز كل من الكالسيوم وانزيم الفوسفاتيز القلوي في الأسابيع التي تلت العملية الجراحية. نستنتج من هذه الدراسة ان استخدام نخاع العظم والليفين الغني بالصفيحات الدموية سرع من شفاء كسر الجزء القاصي لعظم الكعبرة.

الكلمات المفتاحية:الكسر ، عظم الكعبره ، نخاع العظم ، الليفين الغني بالصفيحات الدموية.