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The effects of thyroidectomy on healing fracture in rabbits model

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Abstract

This research aims to investigate the effects of thyroidectomy on fracture healing. The experiment included sixteen male rabbits which allocated randomly into two equal groups. Rabbits anesthetized by premedicated atropine sulphate, then 15 minutes later, a mixture of Xylazine Ketamine was used. The control group rabbits were induced with mid-shafted femoral fracture, with that, it fixed firmly by intramedullary pinning. Meanwhile, the treated group rabbits were induced with same method mentioned above, but fracture were fixed after thyroidectomy and investigation of thyroxin a week postoperatively. In control group results involved sever inflammatory reaction as compare to treatment group. The signs of inflation subsided gradually during 4-5 days in treatment group, whereas, it took 5-6 days in control animals. Also, radiological investigation of both groups exhibited periosteal reaction which was observed at end of 2nd week in the control group, whereas, the treated group showed its reaction at the end of fourth week, there was an invisible fracture line for both control and treated groups at the end of fourth and sixth week, respectively. In addition, bony bridge in the control groups became remodeled into a lamellar bone earlier than treated group. The results of measuring thyroxin hormone before and after the thyroidectomy confirmed the essential role of this hormone in fracture healing. To conclude that the fracture healing maturity was superior in control group than in treated group.

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Introduction

Bones are anatomical structures in the body that perform several important functions; Such as supporting soft tissues, protecting the body's internal organs. Although motor function is still important, maintaining the level of calcium in the blood is also critical because bones are the first source of calcium in the body (1,2). The fractures are one of the challenge difficulties in the area of orthopedic surgery, where a fixation and healing process includes a chain of complex processes in physiologically biochemistry which effects on the body health (3,4). It is greatly observed over the past two decades, that the orthopedic surgeons have dramatically progressed their capability to refurbish the structure, and the functionality of damaged joints and bones. This through studying newly proposed methods of internal fixation, external fixation, and rehabilitation; also, surgeons have been successfully practiced in treating a highly severity fractures (5,6). In contrast to other tissues in adults, when skeletons recover, scar tissue is created at the site of injury by the formation of new bone adjacent to the uninjured tissue so that it is indistinguishable. Aspects of the regeneration process for adults' bear similarities in terms of both embryonic and adult skeletal progenitor cells, as they aggregate to form a cellular condensation, which eventually differentiates into skeletal tissue (7). The early event of fracture healing is the reconstruction of vascularity, and it is up to the regulation of angiogenesis that has a tendency to promote the formation of bone (8,9). The regulation of heart rate (HR) in the cardiovascular system is due to the thyroid activity hormones. the manifestations of hypothyroidism in animals is a low heart rate, when compared to hyperthyroidism which have tachycardia (10,11). Thyroid hormones allow an increment in peripheral oxygen consumption and these substrates requirements that are the secondary cause of an increment to the cardiac contractility (12). In addition, they are able to maintain the inotropic and chronotropic effects on the cardiac functionalities of the body (13). Besides that, the thyroid hormones highly influence the immunological response present in the mammals, where studies stated that the administration of the thyroid hormones induces leukocytosis in the normal animal's species (14).

Therefore, the aim of this study is to evaluate the effects of the thyroidectomy on fracture healing.

Materials and methods

Sixteen mature male rabbits, weighting 1 kg - 6 kg Bwt, were used in this study**.** They were certified as healthy by their clinical authority and physical examination, they were housing under similar condition and feeding also was verified. Before starting any surgery, the concentration of thyroxin hormone was investigated in all experimental animals. Consequently, rabbits were categorized into two equal groups for experimentation: (i) control group, which was experimented with inducing mid-shaft femoral fracture, and then the fracture was fixed by intramedullary pinning, (ii) treated group, on which a mid-shaft femoral fracture was also induced and fixed by intramedullary pinning and thyroidectomy was done and the thyroxin hormone was measured a week post-operation to the animals in both groups. Animals were subjected to fasting for 24 hours and 12 hours of water prior to the procedure. The surgical site which involved the lateral and medial side of thigh, in addition to the neck in the second group were organized under aseptic technique. Atropine sulfate at a dose of 0.05 mg/kg B.W intramuscularly were injected in the animals as premedication, then after a period of 15 minutes, mixture of 2% xylazine, 20 mg/kg B.W and 5% ketamine, 40 mg/kg B.W was given intramuscularly, the half dose was repeated if it essential (15). The animals were recumbent at lateral side, in control group; a longitudinal skin incision was made on lateral side of the thigh between the great trochanter of femur and the lateral aspect of the patella. Consequently, a sharp incision of the fascia, and a blunt dissection between biceps femoris and vastus lateralis muscles was done to expose the femoral bone (Figure 1). Then curved artery forceps was introduced beneath the bone to facilitate the insertion of wire saw for making transverse fracture through the mid-shaft of the bone (Figures 2 and 3). The fracture ends were fixed by intramedullary pinning (Steinman stainless steel 2.4 Q x 120 mm) by retrograde technique (Figures 4-6). Then muscles and fascia were closed by simple continuous pattern, using cat gut size 2.0. Skin was sutured with a simple interrupted pattern by silk size 2.0. Meanwhile in treated group, same procedure was used as describe in control group, except that thyroidectomy was achieved after fixation of two fracture ends. Penicillin-streptomycin at a dose of 10000 IU/Kg Bwt. intramuscular was given for four days after the operation. Lastly, animals were kept under observation during the experiment period and the skin suture was removed ten days after operation. Clinical, radiological investigations and hormonal assay were taken in different periods along the study.

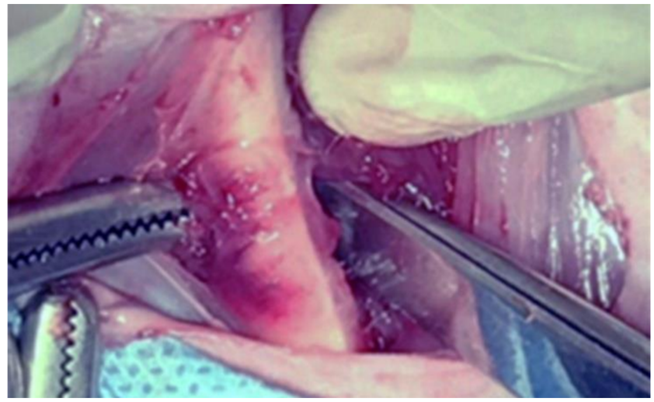

Figure 1: Show expose of the femoral bone.

Figure 2: Show insertion of wire saw beneath the femoral bone.

Figure 3: Show making fracture in the mid-shaft of femoral bone.

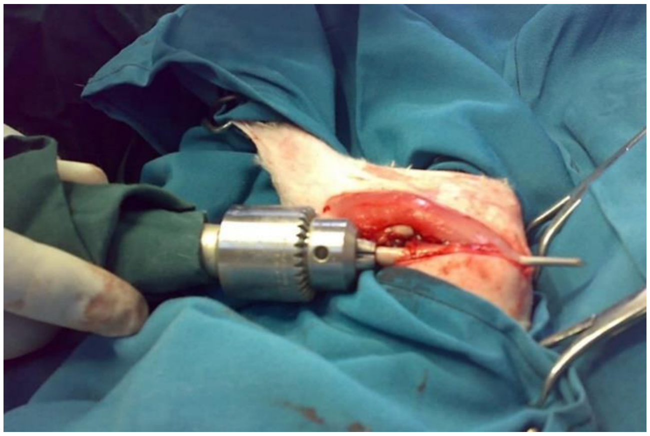

Figure 4: Show retrograde induction of intramedullary pin in bone marrow.

Figure 5: Show induction of pin in distal segment of fracture end.

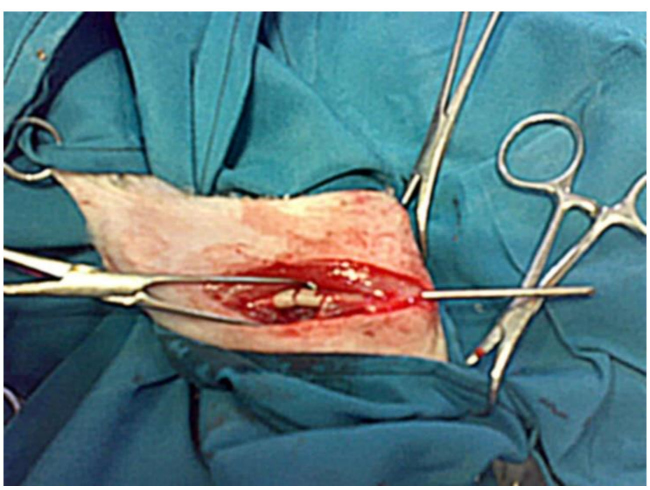

Figure 6: Show complete insertion of intramedullary pin in both fragments.

Statistical analysis

Computer package Sigma plot V12.0 / SYSTAT software was used to conduct the parameter analysis. Data were presented as means ± SE and were analyzed using one-way analysis of variance ANOVA and t-test with the significant level set on $P<0.05$.

Results

Few critical observations were revealed in the results obtained from the clinical examination, in the control group there were inflammatory signs visualized from the 2nd day post-operation. The inflammation includes symptoms of swelling of the surgical site, increased temperature of the area, pain, and the animal is incapable to tolerate the weight on the affected leg. In 5-6 days of post operation, the edema and inflammation had been subsided gradually, and the animal can tolerate the heavy weight of the leg. While, in treated group all above signs were less likely to occur when compared with the control group. These signs which observed in the treated group were visible for up to five days' post operation, and the fracture movement ended at the 4th week.

To assess the degree of fracture healing, X-rays of the fracture sites of both groups was weekly observed. Radiological findings at two weeks post operation in control group demonstrate mild periosteal reaction near the fracture site, with clear fracture line (Figure 7). However, the treated group includes a clear fracture line with a sharp end of the both proximal and distal bone fragments, with clear fracture line (no periosteal reaction nearby fracture site) (Figure 8). As the four weeks' end, an external callus bridge of the fracture site in both sites, with visible fracture line was observed in control group (Figure 9). While in treated group, an external callus was observed around the fracture site with visible fracture line (Figure 10). In control group at fifth week, an invisible fracture line was presented and more prominence of callus formation around the fracture site where anew bone formation in the proximal and distal part is observed; the callus formation from the distal part try to cross the fracture line (Figure 11). While in treated group, an external callus formation bridged the fracture site but not mature enough, a visible fracture line (Figure 12). At the end of sixth week in the control group, the callus formation across the fracture line and try to make bridge between the two fragments with shown in an invisible fracture line (Figure 13), while in the treated group the callus around the fracture site try to bridge the fracture end; however, there was invisible fracture line (Figure 14). In control group at the seventh week post operation, decrease callus size around the fracture site was observed, with an invisible fracture line (Figure 15). Meanwhile in treated group, callus formation from the caudal aspect of the fragment try to cross the fracture line (Figure 16). In eighth week post operation, an attempt of incorporation of the extend callus and contour of the bone itself was observed in control group (Figure 17). In treated group, a decrease in the size of external callus around the fracture site after operation was observed at eighth week post operation (Figure 18). Remodeling process was observed at fracture site, and the bone may be taken back to its normal shape, and external callus decreases in size at ninth weeks in control group (Figure 19). An external callus attempt incorporation with the cortex of the bone itself at ninth weeks in treated group was seen (Figure 20).

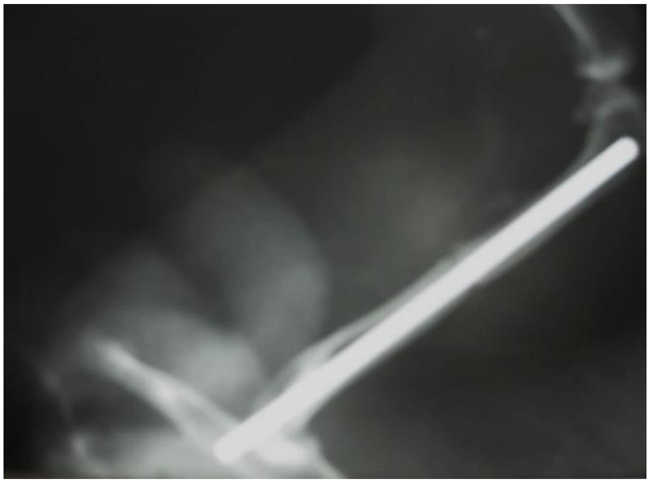

Figure 7: Radiographic image at 2nd weeks post-operation in control group showed minimal periosteal reaction observed around the fracture site, with visible fracture line.

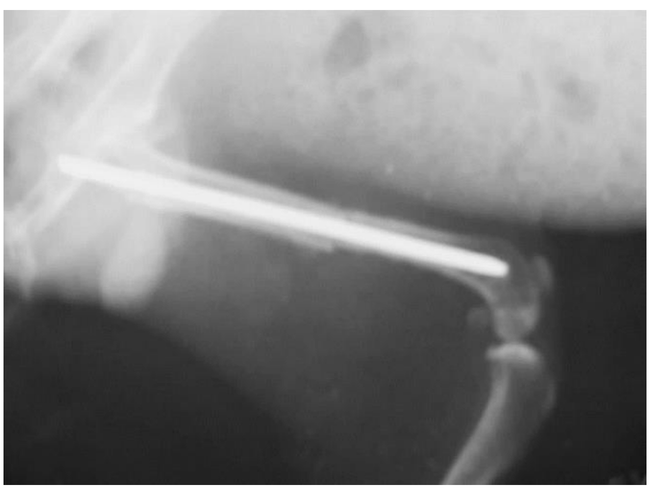

Figure 8: Radiographic image at 2nd weeks post-operation in treated group showed very slight periosteal reaction at the fracture site, visible fracture line.

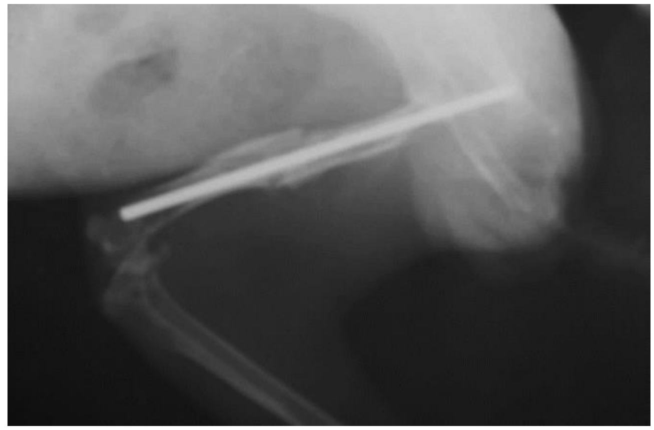

Figure 9: Radiographic image at 4th weeks post-operation in control group showed callus formation around the fracture site in both sites, but still visible fracture line.

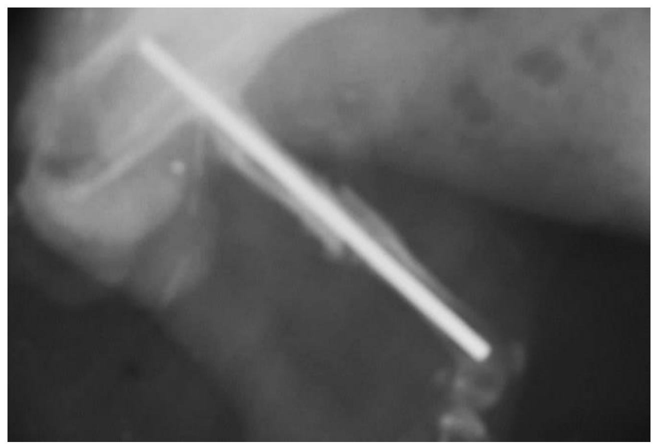

Figure 10: Radiographic image at 4th weeks post-operation in treated group showed minimal callus around the fracture site and visible fracture line.

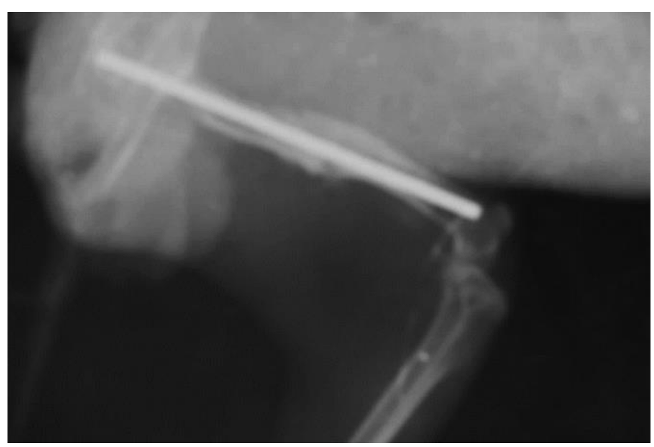

Figure 11: Radiographic image at 5th weeks post-operation in control group showed callus around the fracture site, invisible fracture line.

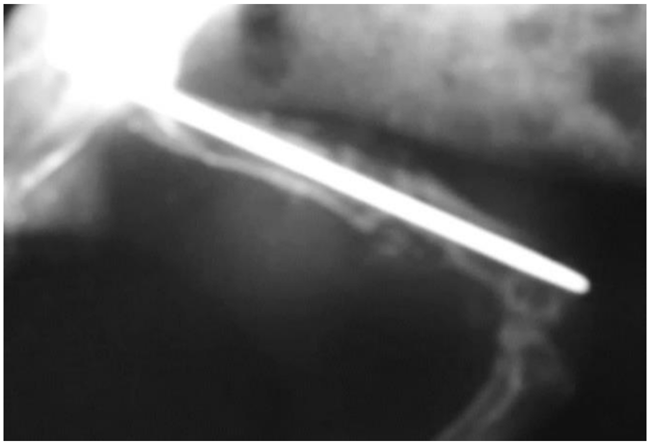

Figure 12: Radiographic image at 5th weeks post-operation in control group showed callus around the fracture site, visible fracture line.

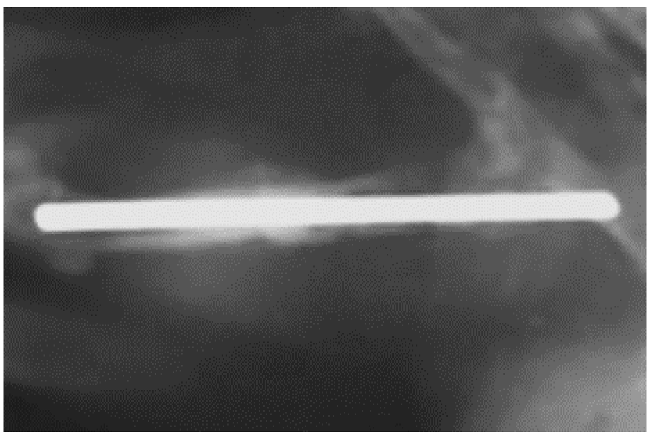

Figure 13: Radiographic image at 6th weeks post-operation in control group showed minimal callus around the fracture site with invisible fracture line.

Figure 14: Radiographic image at 6th weeks post-operation in treated group showed callus formation bridge the fracture end, invisible fracture line.

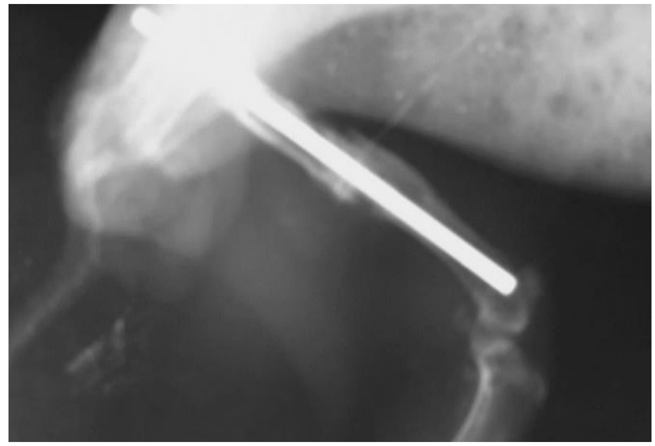

Figure 15: Radiographic image at 7th weeks post-operation in control group showed decrease in the size of callus around the fracture site, invisible fracture line.

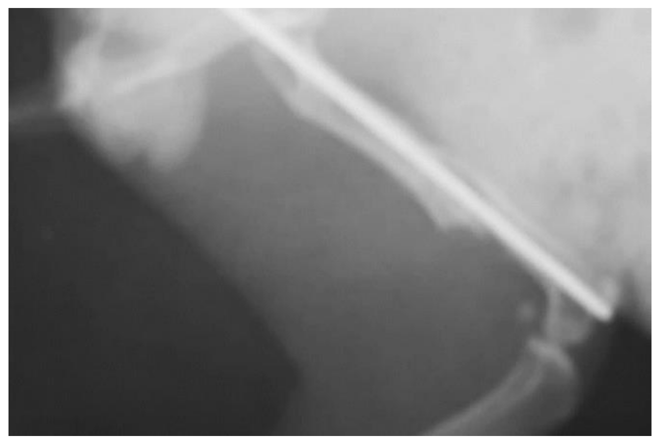

Figure 16: Radiographic image at 7th weeks post-operation in control group showed callus formation from the caudal aspect of the fragment try to cross the fracture line.

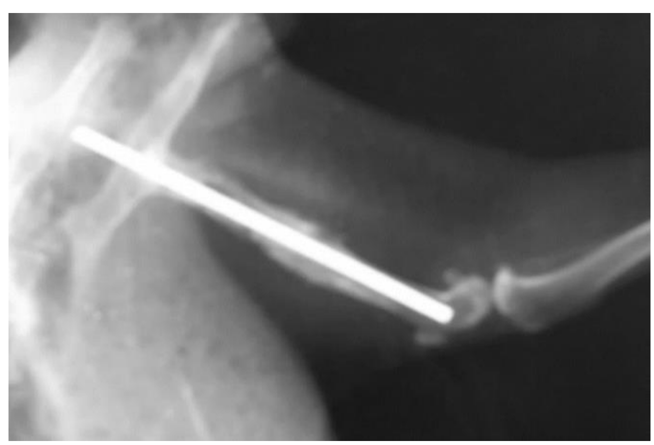

Figure 17: Radiographic image at 8th weeks post-operation in control group showed an attempt of incorporation of the extend callus and contour of the bone itself.

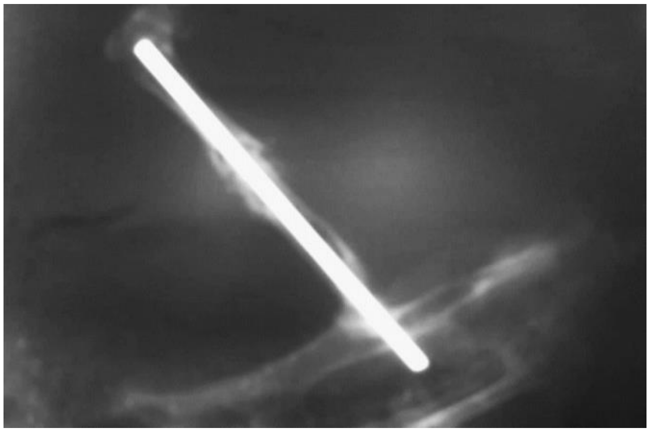

Figure 18: Radiographic image at 8th weeks post-operation in treated group showed minimal callus around the fracture site.

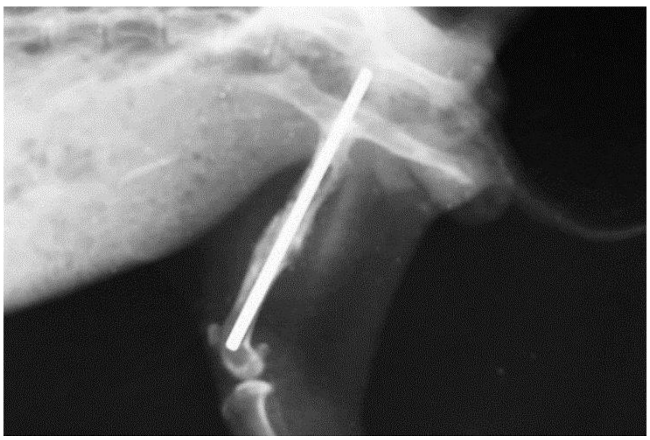

Figure 19: Radiographic image at 9th weeks post-operation in control group showed remodeling process was observed at fracture site, and the bone may be taken back to its normal shape, and external callus decreases in size.

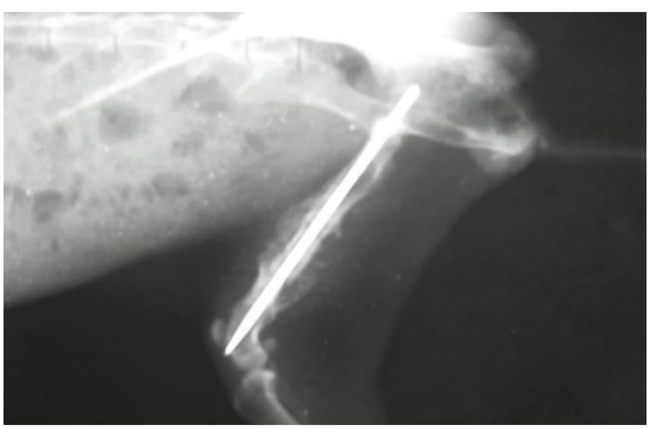

Figure 20: Radiographic image at 9th weeks post-operation in treated group showed external callus attempt incorporation with the cortex of the bone itself.

Hormonal assay in control group shows, there is no significant difference between its values at pre-thyroidectomy 56.23± 14.21 nmol/L as a compare with post-thyroidectomy 49.63± 45.27 nmol/L (Table 1). While, hormonal assay in treated group shows significant difference between its values at pre-thyroidectomy 52.71± 18.01 nmol/L when compared with post-thyroidectomy 0.23± 21.11 nmol/L (Table 1).

Table 1: Thyroxin hormone concentrations pre-operation and post-operation

Groups	Mean (nmol/L) ± SE	
	Pre-operation	Post-operation
Control	56.23 ± 14.21^{a}	49.63 ± 45.27^{a}
Treatment	52.71 ± 18.01^{a}	0.23 ± 21.11^{b}

a, b within each row, averages marked with different letters differ considerably in terms of significance (P≤0.05).

Discussion

In the control group, there was inflammatory signs visualized from the 2nd day post-operation. The inflammation includes symptoms of edema of operation site, increased temperature of the area, pain, and the animal is unable to tolerate the weight on the affected leg. In five to six days of post operation, the edema inflammation had been disappeared. Meanwhile, treated group all above signs were less likely to occur. In addition, the observed signs in treated group were only visible for up to five days after the operation. This may be caused by an increased blood flow in operative area, despite the increased blood vessels dilation with increased capillaries permeability; this has led to the white blood cell migration of inflammatory cells, out of the site of blood vessels with edema formation in operation site. This remark agreed with the researchers in this field of operation (3).

It is observed that the pain may have been caused due to the edema around the fracture area, hence, leading to an increment in the pressure over the nerve endings. Moreover, the higher the inflammation severity, the simulation of cells in production of prostaglandin increases. This is mainly the subsequent that leads to a vasodilatation, thus, leading to an increment infiltration of the accumulated blood vessels of exudation in fracture area. Nevertheless, the inflammatory signs have been vanished within 4-5 days' post-operation in the control animals. Meanwhile, clinical signs in the treated groups, were less severe comparing with control animals. This can be due to the reason of vascular effect of thyroid hormones that produces huge number of phagocytes in operating site; causing as increased severity of inflammation in tissues. Because of thyroid hormones high vascular effects and an appropriate amount of rich source of angiogenic factors that promote the growth of blood vessels into whatever tissue that needs to grow. Furthermore, the factors that are related to both groups of operation, it was observed that the torn blood supply of the bone and its tissues surrounding, white blood cells, and the plasma exudation. Thus, these are caused by oxygen reduction and increase area acidity that causes the place of operation to swell and have inflammation to remove necrotic tissues (4).

The movement of fractured ends which was detected during palpation of fracture site may be the result of an internal, an intermediate, and an external callus; that is not connected with the fracture ends. However, it was observed to be healed by the end of 3rd week in the control group. This finding can be correlated with Malizos and Papatheodorou, and Smit *et al*. (16,17), where the authors suggested that the periosteum contributes in the secondary callus formation with the cells and their growth factors, whilst, it must be preserved and protected during surgery to ensure fracture management is proper. In treated group, this scenario can be mapped as the callus formation around fracture site, with the less prominence than that of control group. These results are observed and occurred due to the thyroid hormones with the decreased metabolism of calcium and bone. With that, the calcium movement that occurs between bone and blood plus the vascularity contains the fibroblasts and lymph cells. These are able to produce the connective tissues surrounding the fracture site and forming of collar from fibrous tissue around the fracture ends. Henceforth, this is able to provide an immobilization of the operated region and protective of new outgrowth capillaries formation was observed. This is a basic important observation to success of fracture healing process, and phagocytes to produce factor growth fibroblast act to attract and stimulate the mesenchymal cells to region and recognized to fibroblast. These were also agreed with other researchers (18).

When the periosteum has been affected due to poor blood supply to the bone due to thyroid hormones decrement; this plays important role in direct stimulation of bone metabolism activity that are carried by some of these osteogenic cells (19). In treated group, at the second week of post operation; there was no reaction of periosteum observed at the fracture site. This may be due to the decreased thyroid hormones that affected on the metabolism of calcium and bone and effected on the fracture site vascularity that effect on the formation of new blood vessels from surrounding tissues. The outgrowth of blood vessels is an important aspect to be formed over the callus surrounding the fracture site; as agreed with Malizos and Papatheodorou, and Luisetto *et al*. (16,20). Meanwhile, this slight reaction of periosteum was observed in control group at the termination of 2nd week after operation.

In treated group at fifth week of post operation; an external callus formation was observed to bridge the fracture site but this bridging was un-mature, so there were visible fracture lines, and this was differed when it compared with the control group on which the fracture line was diapered at the fifth week. New bone formation in the proximal and distal part occurs due to the callus formation from the distal part which tried to cross the fracture line. This occurs due to a decreased in thyroid hormone level which influence on bone healing in different ways. Transforming growth factor is able to regulate angiogenesis, form bones, provides an extracellular matrix synthesis, and also, it has the ability to control cell-mediated activities occurring in the body. Cell attachment can be promoted through the processes such as fibronectin, osteocalcin, osteopontin and osteonectin. This provides a facility for the cells to migrate and activates required cells, called the calcitonin. Furthermore, it also reacts when there is a rise in plasma calcium level. Calcitonin serves to inhibit calcium-dependent cellular metabolic activity, these agreements with Kim *et al*. (6).

In treated group at eight weeks, the external callus wasn't incorporate with the cortex of the bone itself. Meanwhile, in the control group; callus incorporation was slightly observed, this may be due to thyroid hormones that seems to take indirectly stimulating role for metabolic activity of the osteocytes. Therefore, influencing the turnover of calcium in the skeleton. This phenomenon is referred to the resorption of the callus to return the mechanical genitivally of the bone to its normal function, and these were agreed with Riddle and Clemens, and Vammi *et al*. (21,22).

Hormonal assay in control group shows, there is no significant difference between its values at pre-thyroidectomy and post-thyroidectomy. The results of the clinical and radiographic examination showed that the fracture healing was not affected, and it was within the normal rates, and this is due to the stability of the factors affecting the healing, the most important of which is the hormone thyroxin, this is consistent with what Chmurska *et al*. (23) found. Whereas, hormonal assay in treated group shows significant difference between its values at pre-thyroidectomy compared with post-surgery. The healing of fractures was significantly affected in terms of delayed healing sometimes and non-healing at other times, and this was confirmed by the results of the clinical and radiological examination. The most important reason for the delay in healing may be the disappearance of the effect of the hormone thyroxine, which plays a key role in supporting fracture healing, this considered with Lo *et al*. and Reddy *et al*. (24,25).

Conclusion

To conclude this study, it was exhibited that the degree of fracture healing in control group is more prominence than in treated group; and the contour of bone was restored faster in control group than it was in treated group. Thyroidectomy have direct and indirect effect on delay bone healing.

Acknowledgments

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Conflict of Interest

The authors declare that no conflict of interest exists.

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تأثير استئصال الغدة الدرقية على التئام الكسر في الأرانب

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الخلاصة

هدف هذا البحث إلى معرفة تأثير استئصال الغدة الدرقية على التئام الكسور. اشتملت التجربة على ستة عشر ذكرًا حيث تم تقسيمها عشوائيًا إلى مجموعتين متساويتين والتي تم تخديرها باستخدام خليط من الزايلازين والكيتامين. تم تعريض أرانب مجموعة السيطرة لكسر في وسط عظم الفخذ والذي تم تثبيته بإحكام عن طريق التثبيت داخل النخاع. وفي الوقت نفسه، تم تعريض أرانب مجموعة المعالجة لنفس الطريقة المذكورة أعلاه، ولكن تم إصلاح الكسر بعد استئصال الغدة الدرقية. تم فحص هرمون الغدة الدرقية لكلا المجموعتين بعد أسبوع من اجراء العملية الجراحية. تضمنت نتائج مجموعة السيطرة تفاعلًا التهابيًا شديدًا مقارنة بمجموعة المعالجة. تراجعت علامات الالتهاب تدريجياً خلال ٤-٥ أيام في المجموعة المعالجة، بينما استغرقت ٥-٦ أيام في مجموعة السيطرة. أيضا، أظهر الفحص الإشعاعي لكلا المجموعتين تفاعل سمحاقي لوحظ في نهاية الأسبوع الثاني في مجموعة السيطرة، بينما أظهرت مجموعة المعالجة رد فعلها في نهاية الأسبوع الرابع، وكان هناك خط كسر غير مرئي لكل من المجموعتين السيطرة والمعالجة في نهاية الأسبوع الرابع لمجموعة السيطرة والسادس لمجموعة المعالجة. بالإضافة إلى ذلك، تم إعادة تشكيل الجسر العظمي في مجموعة السيطرة ليصبح عظم رقائقي قبل مجموعة المعالجة. أكدت نتائج قياس هرمون الثيروكسين قبل وبعد استئصال الغدة الدرقية الدور الأساسي لهذا الهرمون في التئام الكسور. استنتج من ذلك أن نضج التئام الكسر كان أسرع في مجموعة السيطرة منه في مجموعة المعالجة.