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Effect of Low Level Laser Therapy on the Sheep Ribs Xeno Graft in the Treatment of Rabbits Long Bone Fractures

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Abstract

The aim of this study was to estimate the clinical, radiological, and histopathological effect of the low level laser therapy (LLLT) on sheep ribs xeno graft which used as bone replacement for the treatment of the femoral fractures in rabbits. 20 adults rabbits of local breed were employed to removing about 1cm length of bone from the femoral bone surgically, and replaced the empty space with suitable size of sheep ribs xeno graft which fixed by intramedullary pinning .The animals were divided to ,control group was of 10 rabbits lifted for normal healing processing ,with no laser irradiation post operation (P.O.), while the treatment group of 10 rabbits exposed to single daily dosage of continuous diode laser at four points of the lateral aspect of the femoral bone for 5 minutes at 72th interval for 14th days p.o. laser dosage (850 nm,148.4J/cm2).The experimental period 12 wks p.o.

The clinical observation revealed that the treatment group normally use the affected limb within 14th to 21th days p.o. while it remain after 21th days p.o in the control group, At the end of 12th wks p.o. after removing the intramedullary pins, the femoral bones re-fractured in the control groups, while it remain in the treatment group stable well fixed and not fracture.

The radiological finding showed, early periosteal reaction with new bone formation at the end of the 1st week p.o. in the treatment group which increase in volume and opacity in the 3rd and 4th weeks p.o. to form the bony bridge, while in the control group the periosteal reaction and the new bone formation appears at the end of 2nd weeks p.o., and the bony bridge formation occurs at the end of 6th weeks p.o.

The histopathological finding after 6th wk's p.o. in the treatment groups mature and wide trabecular bone formation. The control group showed thin immature trabecular bone, with large cavities of none organized Connective tissues. At the end of 12th wks p.o.in the treatment groups lamellar bone formation surround the xeno graft, with partial bony incorporation, new blood vessels and osteocytes invade the bony device .compare with the control groups the trabecula bone not yet all mature, and many empty lucana and havesan canal .

The conclusion is the irradiated sheep ribs xeno graft by LLLT, can be used to fill the femoral defect, promote healing,

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and well fixed and tolerate body weight, with no complication or body rejection.

Key words: sheep ribs xeno graft, low level laser therapy, femoral fracture, femoral defect.

Introduction:

Fracture healing is a complex biological procedure and its aimed is to regenerate mineralized tissue, restore the mechanical strength, and normalize the functionality of the repaired tissue (Marsell and Einhorn 2011). Treatment fractures characterized by bone defect or cavities or even loss part of bone, is by Bone grafts/implantations as bone substitution, space filler and source of calcium (LaStayo et al., 2003; Matsumine et al., 2004; Ogose et al., 2005; Walsh et al., 2008; Nazht et al., 2018).Using Bone graft /implantation can used for promote healing, and as a frame work to provide bridge for establishing the continuity of long bone defects (Myerson and Uribe 2003).

repair bone defects in rats, mice, dogs, and sheep done by using seeded bony scaffolds with mesenchymal stem cells of the autologus bone marrow (Kon et al. ,2000) ,or using prostaglandin (PGE_2) to inhance bone reabsorption of the bony tubular splint in the remodeling phase in rabbits (Nazhat, 2000),or enhance bone healing by using electrical stimulation locally (Briggs et al., 2004; Nawrocki et al. ,2006). Or Using the Extracorporeal shock wave to improve fractures healing, (Schaden et al 2001; Ciampa et al., 2005). Recently new technique and methods used to facilitate fracture healing by using Laser, growth factors, and platelets rich plasma (Hallman and Thor, 2008).

LLLT has a benefits effect on the repair bone defect grafted by organic bovine bone (Weber et al., 2006;Marleny et al., 2008), it is widely used for accelerating fracture healing, wound management arthritis(Pinheiro et al .,2003;Lopes et al.,2007), also can be used to enhance callus formation and blood vessels angiogenesis (Shakouri et al.,2010;Merli et al ., 2012). laser can stimulate osteoblasts cells for the healing of lower mandible defect in rabbits (Nazht et al., 2013).Nazht and his group refer in (2016) and (2017) that LLLT inhance fracture healing in the distal third of radius in dogs .

The aim of this study was to investigate the effect of LLLT on the sheep ribs xeno graft which used as a space filler for the treatment of rabbits femoral fracture.

Materials and methods:

20 adult rabbits of local breed both sexes weighting 1.500-1.750 kg were used, the surgical operations were done under general anesthesia with the combination of xylzine hydrochloride 2% in a dose of 17.5mg/kg.B.W. And ketamine hydrochloride 10% in a dose 25mg/kg.B.W. respectively .The skin, subcutaneous tissues and fascia – lata sharply opened, the vastus lateralis and biceps femoralis muscles bluntly dissected, to exposed the femoral bone ,1cm length of the diaphyseal femoral bone removed by electrical saw, cooled the bone by dripping sterile normal saline, the empty space filled with suitable size of sheep ribs xeno grafts (prepared from the sheep ribs after cleaning deprotenization and sterilization) fixed with intramedullary pinning. The muscles and the fascia- lata sutured by 2/0 absorbable sutures materials, the skin closed with 2/0 non absorbable sutures materials .The operated animals divided to two groups each contain 10 rabbits .The control groups lifted for normal healing process without laser irradiation, while the treatment group exposed to a single daily continuous diode laser at 850 nm at 4 point at the lateral aspect of the thigh region for 5 minutes at 72 hours interval at the dose (148.4 J/cm^2). Daily intramuscular systemic antibiotics injection of penicillin streptomycin for 3days p.o. the parameters were daily clinical observation until the end of 12weeks p.o. and weekly radiographic finding finally the histopathological examination at the end of 6^{th} and 12^{th} week's p.o.

Results:

The clinical observation, revealed body depression, difficult in movement and loss appetite in both groups first day p.o, then returned normally 3 to 5 days p.o. ,the skin incision healed satisfactory 5-7 days p.o. with no complication ,the treatment group normally used the limbs 14^{th} to 21th days p.o, while the control group its remain after the 21th days p.o. At the end of 12^{th} week's p.o. when removing the intrmedullary pinning the limb of the treatment group well fixed weight bearing, and not fractured while the limb in the control group re-fractured.

The radiological finding, shows new bone formation at the end of 1^{st} wk p.o in the treatment group (Fig.1A and B), while the control group it appear at the end of the 2^{nd} wk p.o. (Fig.2 A and B),at the end of 3^{rd} and 4^{th} wks p.o. increase the size and opacity of the callus formation in treatment group and bridge formation occurs to joined the femoral fragments and circle the xeno bony implantation, while the control group appears at the end of the 6th week p.o. (Fig.3 A and B),the remodeling phase continuous until the end of the 12th wks p.o. (Fig.4 A and B), after removing the intramedullary pin the control group re-fractured while the treatment group stable and not fractured (Fig.5 A and B).

The histopathological finding at the end of 6th weeks p.o. mature wide, well mineralized trabecula bone formation in the treatment groups, while in the control groups, thin and less mineralized trabecula bone formation with large cavity (Fig.6 A and B).At the end of 12th weeks p.o. lamellar bone formation in the treatment group circling the bony device with partial bony incorporation ,the empty lacuna of the bony device filled by osteocyts and blood vessels invade the haversian canal ,while the control group thin, less mineralized trabecula bone with many cavity, not organized, with empty lacuna in the bony device,(Fig.7 A and B).

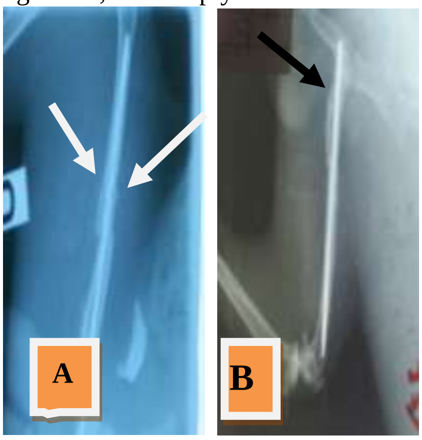

Fig.1 end of 1st wk p.o., A treatment group new bone formation white arrow .B control group, no periosteal reaction black arrow.

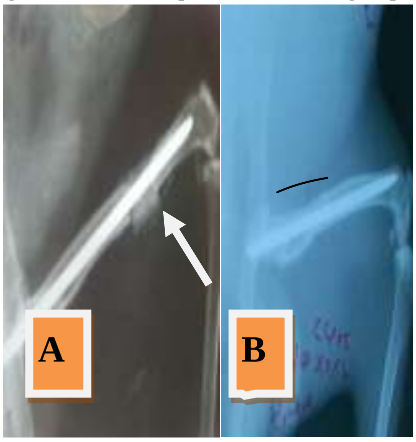

Fig.2 end of 2nd wk p.o. A treatment group, increase callus formation with high radiographic opacity white arrow .B control group, the new bone formation curved line.

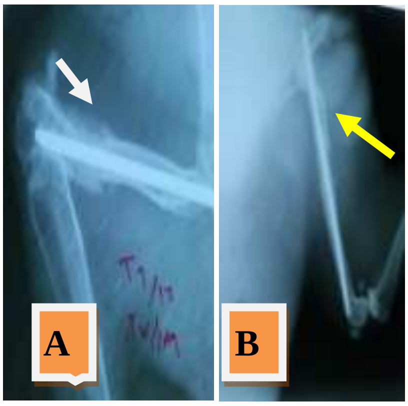

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Fig. 3 end of 4th wk p.o. A. treatment group bridge formation whit arrow .B. Control group, bridge not finished yelow arrow.

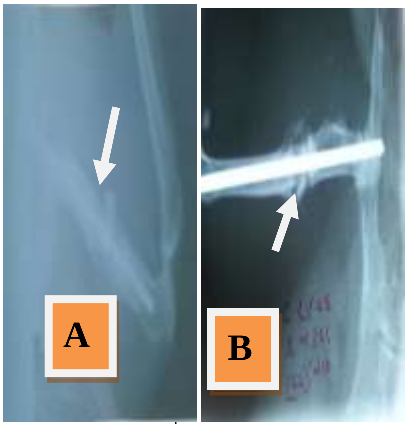

Fig .4 end of 12th wk p.o. .A treatment group and B control group remodelling phase ,sclerotic area at the edge of bony implantation white and black arrow.

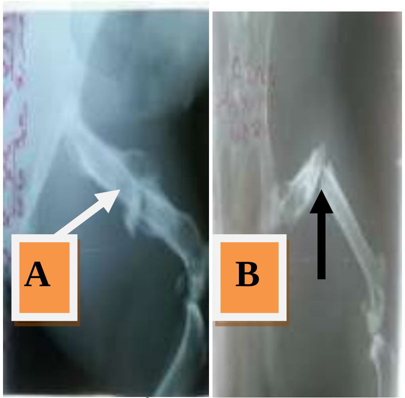

Fig. 5 end of 12th wk p.o.removing the intramedullary pinning.A treatment group femoral bone not fractured white arrow.B control group femoral re-fractured black arrow.

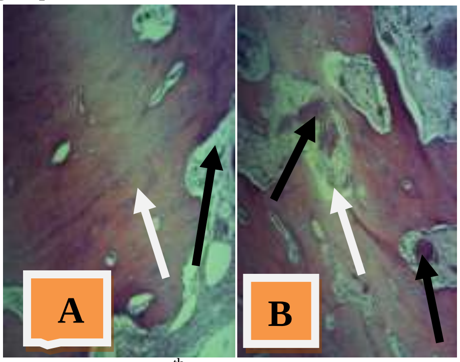

Fig.6 the end of 6th wk p.o. A treatment group mature trabecular bone white arrow lined ,active osteoblast black arrow .B control group trabecular bone, connective tissues white arrow,blood vessels black arrow (H&E×40).

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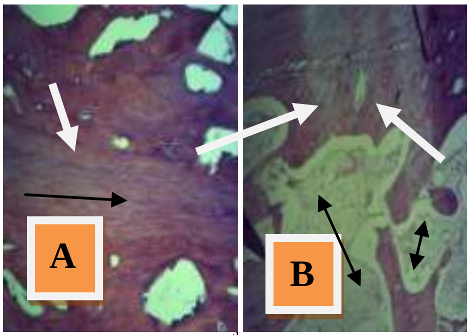

Fig.7 the end of the 12th wk p.o. A. treatment group lamellar bone partially incorporation white arrow, the lacuna and havesian canal filled with asteocytes and blood vessels black arrow .B. control group the mature trabecula bone white arrow, large cavities black double head arrow (H&E×40).

Disussion:

The clinical observation in both groups during the inflammatory phase were normally as mentioned by (LaStayo et al., 2003).The animals move and support the weight after 14 to 21 days p.o. in the treatment group while it remain in the control group untile the end of 21th days p.o., this statement agree with (Singh et al., 2000) that the lameness or difficult in movement disappear gradually in case the fracture getting stabile. The implantion device well placed, well accepted and tolerated by the animals, with no serious inflammation in the surrounding tissue. With satisfactory healing of skin incision with no body rejection toward implantion, all these finding corroborated with (Singh et al., 2000; LaStayo et al., 2003). At the end of 12th weeks post operation in the treatment group when removing the intramedullary pinning, the limb still fixed, not fractured and the animal can use the limbs and support the body weight ,while in the control group the affected limb re-fractured when removing the intramedullary pinning. Because laser irradiation which increase callus formation and promote mineral deposition around the bony device by osteoblastic activity , with improvement in biomechanical properties of the healing bone, as mentioned by (Prado-Fillo and steman 2004; Lirani–Galvaa et al.,2006;Shakouri et al.,2010). (Dortbudak et al., 2000; Cerqueira et al .,2007)refer that low Laser irradiation stimulating mineralization in the early stage of new trabecular bone formation of created bony defects ,and that was clear radiographically at the end of the 1st week p.o. in treatment group

then the callus formation increase in volume and opacity within the 2nd and 3rd weeks p.o.(Khadra et al., 2004;Stein et al .,2005;Weber et al ., 2006;Pretel et al .,2007; Tajali et al ., 2010), they declare that the high radiographic opacity at the end of the device, due to stimulation of laser therapy on the osteoblast activities, while in the control groups the new bone formation started to recognized radiographically at the end of the second weeks p.o. ,and gradually increased slowly next wks. This agree with (Lirani–Galvaa et al., 2006) that the periosteal reaction normally appear radiographically from 10to 14 days p.o.

The laser results in acceleration numbers and metabolism of fibroblast which lead to produce greater number of collagen fibers that will later lead to change to mature trabecula bone, in treatment group compare with control group at the end of 6th weeks p.o.(AL-Watban and Zhang 2001;Schindeler et al ., 2008).

Many of The empty lacuna in the bony device not filled completely with osteocyte in control group and this statement agree with (Dortbudak et al., 2000; Lirani –Galvaa et al., 2006) that 70% of the bony device which implanted not changes to active or alive bone. while In the treatment group the LLLT accelerates maturation of new bone tissue, by enhancement bone metabolism and mineralization during early bone healing, by the stimulate of bone formation and opacity (Schindeler et al., 2008;Marsell and Einhorn 2011).

Conclusion:

The irradiated sheep ribs xeno grafts with LLLT, quickly changed to active and alive bone, can promote fractures healing and repair the bone defect with partially bony incorporation with the recipient femoral bone, and strongly support the body weight, with no body rejection.

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