Dental implants are bioincompatible screw like titanium objects that are surgically placed into the mandible or maxilla to replace missing teeth. The mechanism by which an implant is biomechanically accepted by the jaw bone is called osseointegration.

The clinical long-term success of the implants depends on the osseointegration and the adhesion of the soft tissues and epithelium to the titanium surfaces of the implant.[3]

Titanium is the most wide spread metal for orthopedic implants intended for bone integration.

Results: Immunohistochemical findings revealed high positive expression for VEGF and TGF-β in experimental implant in comparison to control one and the acceleration of bone formation and increase rapid healing process in the screw implant with laser irradiation than in the control implant. Removal torque test showed dramatic increase with the presence of laser irradiation especially with advancing time.

Conclusion: This study illustrated that the LLLT applications enhance bone formation and increase oseointegration.

Key words: Dental Implants, low level laser therapy, Biochemical bone marker.
of fractures, fast recovery from the damage of soft tissue, nerve, bone, and cartilage, and relieves acute and chronic pain and inflammation \(^{(6)}\).

Transforming growth factor-beta (TGF-β), the largest source of which is bone, has been implicated in osteoblast proliferation and differentiation and is expressed at high levels during bone growth and development with an adequate blood supply \(^{(7)}\).

Vascular endothelial growth factor (VEGF), which is secreted by many cells including osteoblasts and osteoblast-like cells, plays an important role for adequate angiogenesis and may be intimately related to bone development and fracture healing because both intramembranous and endochondral ossifications are associated with capillary development. These two proteins are associated with osteogenesis during bone growth, development, and healing; but they do not stimulate stem cells or bone progenitor cells to generate to be osteoblasts as directly as bone morphogenetic proteins (BMPs). However, these proteins have efficacy on not only cell migration and propagation but also on angiogenesis indispensable for bone formation \(^{(7)}\).

**MATERIALS AND METHODS**

Thirty-two adult New Zealand white rabbits male weighing 2-3kg were used in this study, screw titanium implants inserted in the tibia under general anesthesia. The right side is considered as experimental groups and the left side considered as control groups. Low power diode laser (GaAlAs) with wave length (904nm) and (5mW)power applied with the right screw implants. The sample divided into four groups, eight rabbits are sacrificed at four interval 4days, 1 weeks, 2weeks, and 6weeks respectively. Immunohistochemical (VEGF&TGF-β) were done for each interval with mechanical test in 2 and 6 weeks.

**RESULTS**

**Expression of VEGF findings**

At 4 days duration

**Control group**

Immunohistochemical findings of implant site shows positive expression of VEGF in bone marrow stromal cell Figure (1).

**Experimental group**

Immunohistological localization of VEGF in rabbit tibia shows strong positive expression of VEGF in bone marrow stromal cell with the formation of primitive osteoid tissue Figure (2).

At 1 week duration

**Control group**

Woven bone are formed at implant site with weak positive expression of VEGF in progenitor cell, extracellular matrix, blood vessel and osteoid tissue, fat cell shows negative expression of VEGF. DAB stain with hematoxylin counter stain X200. Figure (3).
Experimental group

Positive immunohistochemical localization of VEGF is viewed in large area of newly formed woven bone, endothelial cell of blood vessel, osteoblast cell and active osteoprogenitor cell. Figure (4).

At 2 week duration

Control group

Microscopic evaluation of bone section at implant site shows osteoid tissue formation that is positively expressed by VEGF Figure (5).

At 6 week duration

Control group

Microphotograph view in rabbit tibia shows positively stained immature bone formation with numerous osteocyte that are irregularly scattered in the bone Figure (7).
Oral Diagnosis

Immunohistochemical

Experimental group
Bone section at implant site shows positive localization of VEGF in mature bone, bone trabeculae appear with lamellated bone, osteocyte are arranged regularly inside bone matrix, and blood vessel within bone trabeculae Figure (8).

Expression of TGF-β findings
At 4 days duration
Control group
Immunohistochemical view revealed primitive new bone formation in which future bone is formed as embryonic type. This bone is characterized by presence of progenitor cells that are scattered randomly which shows positive expression of TGF-β Figure (9).

At 1 week duration
Control group
Implant site of 1 week duration shows positive expression of TGF-β in osteoid tissue, fat cell, endothelial cell, progenitor cell with extracellular matrix Figure (11).
Experimental group
Osteoid tissue shows positive localization of TGF-β in osteoid tissue, fatcell, endothelial cell and in progenitor cell, all are irregularly arranged within primitive bone formed at implant site Figure (12).

Figure 12: View of implant site at one week duration treated with laser irradiation shows positive expression of TGF-β in osteoid tissue (OT), and progenitor cell (PG) DAB stain with hematoxylin counter stain X200.

At 2 weeks duration
Control group
Microscopic evaluation of the bone section related to implant shows bone thread with bone trabeculae that are negatively stained enclosing area of woven bone Figure (13).

Figure 13: Immunohistochemical localization of TGF-B in bone trabeculae (BT) of thread region in implant site of 2 weeks duration (control) DAB stain with hematoxylin counter stain X100

Experimental group
View of rabbit tibia with implant shows positive localization of TGF-β in osteoid tissue, bone trabeculae, osteocyte cell and in marrow tissue Figure (14).

Figure 14: positive immunohistochemical localization of TGF-β in osteoid tissue (OT) and in marrow tissue (MT) in implant site treated with laser irradiation for 2 weeks duration while basal bone (BB) is negatively stain DAB stain with hematoxylin X200.

At 6 week duration
Control group
Positive localization of TGF-β in immature bone deposited at implant site in marrow tissue Figure (15).

Figure 15: Immunohistochemical localization of TGF-β in immature bone (IMB) at implant site (control) after 6 weeks of implantation. DAB stain with hematoxylin counter stain X200

Experimental group
Positive expression of TGF-β in mature bone deposited at implant site, it shows havarsian bone, marrow tissue, osteocyte cell embeded in bone matrix, reversal line that separated old bone from new bone Figure (16).
Mechanical testing

Figure (17) shows the summary statistics of the removal torque value of CPTi implants (control and experimental) after two and six weeks of implantation times, the torque value needed to remove all the implant was higher at six weeks healing period for both control and experimental groups.

After 6 weeks of implantation there was an obvious increase in the means values of the torque force that were needed to unscrew the implants.

The mean torque values for the implants control group was (22.75 n. cm) the highest torque mean value was obtained with implants treated with laser irradiation (25.75 n.cm).

Bone marrow stromal cells (BMSC)

From the obvious findings we can noticed that, there was decrease in BMSC score mean values of positively stained cells for both VEGF and TGF – beta, during the 4 days, 1,2 and 6 weeks of healing intervals concerning control group while the experimented group a slight increase in VEGF score at 2and 6 week period, whereas the mean values of scores of TGF beta showed decrease in 2 and 6 weeks of healing intervals, as shown in Figures (18, 19).

Bone cell

The results of bone cells in present study are illustrated in Figure (20). The majority of the bone cells parameter was reported at the VEGF and TGF beta during healing periods, had been decreased down stair sequentially by the times periods passed of the studied trials. In addition to that, the comparisons significant among different periods of times after treatment reported anon significant differences at P>0.05 due to different markers, and the same statistical results were obtained due to comparisons significant among different periods of times in each groups ( control and experimental )at p> 0.05.
J Bag

recruiting osteoblast progenitors and stimulating TGF-β

Therefore our results record positive expression of differentiation and osteoclast recruitment chondrocyte differentiation, osteoblast as aspects of bone development, including only in bone angiogenesis, but also in various mediators during these processes. It is involved not intramembranous ossification acts as an essential bone maturation around the implant positively.

This study affects osseointegration formation and low-energy laser therapy (in the dose given in this study) affects osseointegration process. The increased removal torque values for lased implants were noted around the implants site. All implants were stable during healing periods in the sense that they could not be removed with manual force without the aid of the torque gage instrument as observed from the results of (8).

The increased removal torque values for lased group comparing with control group indicates that low-energy laser therapy (in the dose given in this study) affects osseointegration formation and bone maturation around the implant positively. This result was in agreement with (9-11)

The present result based on application of LLLT with implant, LLLT creates a number of environmental conditions that appear to accelerate the healing of bone (12). LLLT-related effects include stimulation of blood flow, recruitment and activation of osteoblasts, osteosynthesis, a decrease in osteoclastic activity and anti-inflammatory action (13) could also be considered as factors that stimulate biomaterial osseointegration.

VEGF induces the proliferation, differentiation and migration of vascular endothelial cells and enhances their survival by preventing their apoptosis; it also increases the permeability of the capillaries (14). VEGF works in both processes of endochondral ossification and intramembranous ossification acts as an essential mediator during these processes. It is involved not only in bone angiogenesis, but also in various aspects of bone development, including chondrocyte differentiation, osteoblast differentiation and osteoclast recruitment (15). Therefore our results record positive expression of VEGF in experimental group at 6 weeks duration. TGF-β increases bone formation mainly by recruiting osteoblast progenitors and stimulating their proliferation, thus expanding the pool of committed osteoblasts, as well as by promoting the early stages of differentiation (bone matrix production). On the other hand, it blocks later phases of differentiation and mineralization (16,17).

TGF-β increases the pool of osteoprogenitors both by inducing chemotaxis and proliferation (18).

The present study suggests for beneficial use of LLLT in practice of dentistry implantation or in other branch related to osseointegration process.

REFERENCES


