Histological evaluation of osseointegration around titanium implants in thyroidectomized rabbits (experimental study).

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ABSTRACT

Background: Thyroid hormones are essential for linear growth and peak bone mass acquisition. Hypothyroidism occurs when the thyroid gland produces less than the normal amount of thyroid hormones. The present study was carried out to evaluate the effect of hypothyroidism on osseointegration around the titanium implants screwed in rabbit's tibia.

Materials and methods :Fifty four machined surface Iraqi implants were inserted in 27 male rabbits (2implants in each rabbit's tibia).Eighteen of these rabbits were subjected to near total thyroidectomy to induce hypothyroidism three weeks before implantation surgery. While the remaining 9 rabbits were remain as a control group. Blood sample was taken from each animal at the beginning of this study in order to find the normal range of T3,T4,and TSH .And another blood sample was taken for experimental groups to find the levels of T3,T4,and TSH three weeks after thyroidectomy in order to assess the hypothyroidism status .After 2, 4, 6 weeks after implant surgery (6rabbits from experimental group and 3rabbits from the control group) were sacrificed. In the day of scarification, one of the screws was unscrewed with a torque meter, and the peak torque required to shear off the implant was recorded. Then the decalcified sections of the bone around the implants were studied histologically and histomorphometrically .The eye piece reticule was used for morphometrical studies, which were includes: number of osteocytes, number of osteoblasts, thickness and number of bone trabeculae, and thread width

Results:The results showed that hypothyroid rabbits had delay in osseointegration, bone formation and maturation around implants in almost all rabbits in experimental groups. While the rabbits in the control groups showed improvement in osseointegration around titanium implant. Removal torque test illustrated higher torque test value in control animals than in experimental one. Moreover, there were increases in torque test values in both groups with time. Biochemical serum analysis revealed a decrease in T3, T4, and increase TSH levels in experimental animals.

Conclusion: It can be concluded that there were low bone quality with a delay in bone healing around titanium implants in hypothyroidied rabbits compared with healthy one.

Key words: Hypothyroidism, Titanium implants, Rabbits tibia, Osseointegration. (J Bagh Coll Dentistry 2012;24(2):75-79).

INTRODUCTION

Dental implant treatment has revolutionized oral rehabilitation in partially and fully edentulous patients. When the concept of osseointegration was introduced in relation to titanium endosseous implants [(1)].It became possible to achieve high success rates in association with this treatment modality, and multiple investigations have demonstrated an excellent long-term prognosis**.**

The achievement and maintenance of osseointegration are highly dependent on bone quality and quantity. The systemic conditions may be correlated with impaired bone healing around titanium implants, especially in metabolic bone diseases such as osteoporosis, diabetes mellitus, and hypothyroidism [(2)].

Bone is a highly metabolically active tissue in which the processes of osteoblastic bone formation (anabolic activity) and osteoclastic resorption (catabolic activity) are continuous throughout life.

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Therefore, the capacity of bone tissue to respond to injuries such as fracture or implant placement is associated with several mechanisms and may be affected by different conditions [(3)].

Thyroid hormones are the major regulators of bone metabolism and development. Hypothyroidism is a condition in which the thyroid gland does not make enough thyroid hormone (A deficiency of thyroid hormone) to meet the body's needs. Without enough thyroid hormone, many of the body's functions slow down. The scientific consensus is that untreated hypothyroidism causes an abnormally decreased bone density coupled with poor bone quality, and have been linked to altered osteoblast and osteoclast activity, leading to an imbalance in bone turnover [(4).]

Although thyroid dysfunctions may affect bone metabolism via their effect on thyroid hormone levels that influence bone turnover [(5)] there is a lack of information regarding the effect of changes in T3 and T4 serum levels on bone healing around titanium implants. Thus, the objective of this study was to clarify the establishment and maintenance of

osseointegration in thyroidectomy –induced hypothyroidism in rabbit's tibia.

MATERIALS AND METHODS

Twenty seven male adult New Zealand white rabbits aged from 9 to 12 months were used as animal model in this study. Their weights ranged between 1.5 to 2.5 kg .The animals were kept under the supervision from staff of the animal's house of the College of Veterinary medicine. The animals of this study were divided into two groups, experimental group (18 rabbits) and control group (9 rabbits) .The animals of experimental group were subjected to near total thyroidectomy to induce hypothyroidism, three weeks before implant operation.

The levels of thyroid hormones (T3, T4, and TSH) were detected before and three weeks after the thyroidectomy operation [6].

After 2, 4, 6 weeks intervals, the most distal screw was exposed and unscrewed with a torque meter, and the peak torque required to shear off the implant was recorded.

Then the decalcified sections of the bone-implants block were stained with (H&E) and Van-Gieson's stains for histological and histomorphometrical studies, which were includes: number of osteocytes, number of osteoblasts, thickness and number of bone trabeculae, and thread width

The statistical analyses were calculated by SPSS (personal computer) [7]**.** In all multiple comparisons significant p-value was at ($p< 0.05$).

RESULTS

Torque removal test

The lowest mean torque values for both groups were recorded in 2 weeks while the highest mean value was detected in 6 weeks. Also there was a significant ($P\leq0.01$) decrease in the torque values of the experimental groups compared with their controls for healing period (Table. 1**).**

Table 1: Torque test values of different groups

group / H.P	control	experimental	P- value
2weeks	9.83±0.50	4.77±1.16	P≤0.01*
4weeks	18.83±1.17	10.0±0.38	P≤0.01*
6weeks	23.44±0.78	14.0±1.4	P≤0.01*

Histomorphometrical analysis

Trabicular thickness and number

The results showed that there were significant($P\leq0.01$) decrease in the trabecular bone thickness in experimental group compared to their control in the 6th weeks interval only (Table. 2**).**While there were no significant differences in the trabecular number between the experimental and control groups in all healing periods (Table .3).

Table 2: Trabecular bone thickness (µm)

group / H.P	control	experimental	P- value
2weeks	4.75±0.76	3.75±0.72	N.S
4weeks	10.31±1.29	9.16±0.42	N.S
6weeks	14.06±1.07	9.375±0.81	p≤0.01*

Table 3: Trabecular number in different groups

group / H.P	control	experimental	P- value
2weeks	2.75±0.5	2.60±0.05	N.S
4weeks	5.33±0.76	4.83±0.61	N.S
6weeks	3.40±0.79	3.0±0.91	N.S

Number of osteocytes

The results denote that there was a highly significant($P\leq0.01$)decrease in the number of osteocyts in 2 weeks of the experimental group but there was a significant($P\leq0.05$) increase in the number of osteocyte in the period of six weeks in the experimental group compared with the control group (Table .4) .

Table 4: Osteocytes number in different groups

group / H.P	control	experimental	P- value
2weeks	27.33±1.76	16.33±2.60	P≤0.01**
4weeks	31.67±3.3	28.60±2.1	N.S
6weeks	21.67±0.9	26.75±2.21	P≤0.05*

Number of osteoblast:

The results showed that there is there was a highly significant decrease ($P\leq0.01$) in the number of osteoblasts in the experimental groups as compared with their controls in all healing periods (Table. 5).

Table 5: Number of osteoblast in study groups

group / H.P	control	experimental	P- value
2weeks	29.5±1.56	26.33±0.72	P≤0.01*
4weeks	25.66±1.04	22.66±1.53	P≤0.01*
6weeks	20.33±1.26	16.33±1.53	P≤0.01*

Thread width

The results indicate that there was a significant reduction in thread width of experimental animals of 4 and 6 weeks healing periods when compared with their controls (Table .6) .

Table 6: Thread width in study groups (µm).

group / H.P	control	experimental	P- value
2weeks	18.125±2.13	17.5±1.44	N.S
4weeks	21.875±1.88	18.75±7.2	P≤0.05*
6weeks	26.5±1.27	20.625±0.63	P≤0.01**

Histological findings:

The histological findings in 2-weeks interval showed large and numerous bone trabeculae in control group, while there was few and small bone trabeculae in the thread of experimental group **(Figures 1,2).** The histological appearance of 4-weeks interval revealed immature bone which almost fill the whole thread in control group **(Figure.3**), while in the experimental group of the same period showed that there was fibrous connective tissue in near the implant surface with immature bone **(Figure.4,5).**

The histological picture of 6-weeks interval of the control group illustrated mature bone with numerous incremental lines that fill the whole thread of cortical bone region**(Figure 6**), and thick bone trabeculae near the implant surface in the bone marrow region **(Figure 7).** While the picture of experimental group of the same period revealed still immature bone that fill the whole thread in the compact bone region **(Figure 8)**.

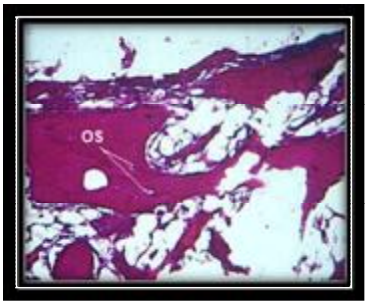

Figure 1: Control group at 2weeks duration showing osteocytes(OS) inside bone trabecule (Van-gieson's stain X200).

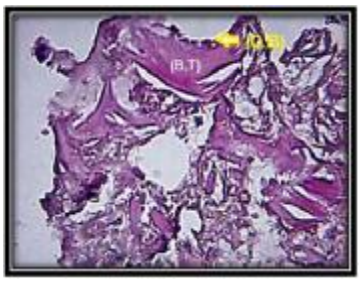

Figure 2: Experimental group at 2weeks duration showing newly formed bone trabecule(B.T) (Van-gieson's stain X 200).

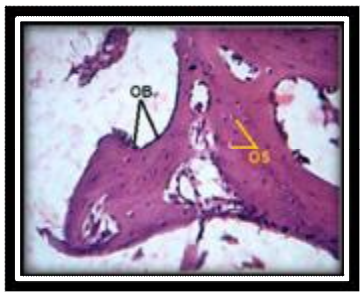

Figure 3: Control group at 4weeks duration showing osteoblast (OB), osteocytes(OS).(H&E X200)

Figure 4: Experimental group in 4weeks duration Showing preosteocytes (POS),osteoclast(OC) (H&E X200).

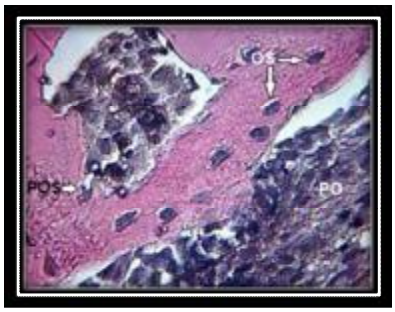

Figure 5: Experimental group at 4weeks duration showing immature compact bone with large size osteocytes (OS), preosteocytes(POS) (Van-gieson's stain X400).

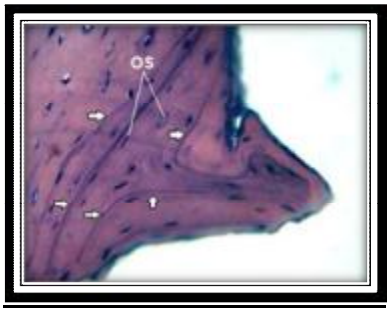

Figure 6: Control group at 6weeks duration .Revealing mature bone thread with numerous incremental lines (arrows) (H&E X400).

Figure 7: Microphotograph of the control group at 6weeks duration revealing bone trabecule in bone marrow region Outline the thread (Van-gieson's stain X200).

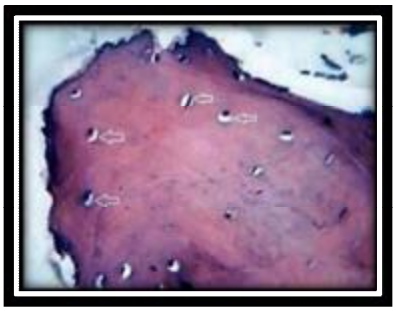

Figure 8: Experimental group at 6 weeks showing immature bone with large size osteocytes (arrows) (H&E X200).

DISCUSSION

This study showed increase in removal torque value over time for both experimental and control groups. It has been suggested that this increase depends on increasing bone-to-implant contact with time as a result of progressive bone formation and maturation around implant during healing, which substantially improved the mechanical capacity [(8)].On the other hand, the increased removal torque values for control animals comparing with hypothyroid animals in all healing periods indicated that hypothyroidism may affect the bone formation and maturation around the implants negatively [(9)].

Histomorphometrical analysis:

T3 regulates the differentiation of osteoblasts, by increasing the expression of many genes of the osteoblastic phenotype like osteocalcin, osteoprotegerin ,and this may explain the decreased number of osteoblasts in the experimental groups in all the study periods compared with their controls [(10).]

Because of ostecytes are derived from osteoblasts[(11)] , the effect of thyroid hormones reduction on osteoblasts can be seen in osteocyte numbers in the experimental groups. Also this study showed decrease in trabecular thickness and numbers in the hypothyroid animals than controls .This most probably due to low bone turnover in hypothyroisim which affect both bone resorption and bone formation and cause reduction in osteiod apposition [(12)].

Hypothyroidism resulted in less newly formed bone within the implant threads and this may explain the reduction in thread width of new bone formed around the implant screwed in the experimental animals. This finding agree with Wilkins et al[(13).]

Histological and histochemical findings:

The histological finding of control rabbits of 2weeks interval showed newly formed woven bone with new bone trabeculae .While the hypothyroid rabbits showed generalized delay in bone remodeling in comparison with control rabbits. This finding may be due to the fewer number of active osteoblasts in the hypothyroid rabbits which are responsible for the formation of new bone matrix. This finding agrees with Williams [(14)]

The histological picture of control animals at 4weeks duration manifested dense newly formed bone rather than trabecular appearances, and osteocytes were trying to get concentric arrangement around haversian canal. While in experimental group the newly formed bone had a trabecular appearance. Osteocytes still irregularly arranged. Osteoclasts and reversal lines were widely seen in the newly formed bone which may gave the indication of continuous bone remodeling. These differences might be attributed to the decrease in the secretion of T3 and T4 in hypothyroid animals which cause delay in bone formation and maturation .This result correlate with previous study done by Williams [(15).]

The histological and histochemical findings of the control animals at 6weeks duration showed almost mature newly formed bone threads .It had the same mature appearance of the original bone .while in experimental animals of this period, the newly formed bone in general was not completely

mature and the osteocytes still irregularly arranged. These slight differences in bone healing between experimental and control in histological and histochemical findings may be due to the effect of hypothyroidism on bone healing around titanium implants [13,15].

It was also shown that an imbalance in the levels of T3 and T4 correlated positively with the levels of the factors involved with bone homeostasis. For instance, a decrease in osteoprogenitor cells, growth factors, and cytokines, resulting in a decreased bone apposition, was reported for hypothyroidism [16].

The present study was done to evaluate the effect of thyroid hormones on bone healing around titanium implants in thyroidectomized rabbits. The findings presented here clearly demonstrate that clinicians should not underestimate these conditions when dealing with patients diagnosed with hypothyroidism that are referred for implant placement.

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