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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL (IGF-1R) EVALUATIONS OF THE EFFECT OF THYRODOCTOMY ON PERIODONTIUM HEALING IN RABBITS

Nada M. H. Al-Ghaban
Oral Histology & Biology, College of Dentistry, Baghdad University, Iraq
* Corresponding author email: nada_gaban@yahoo.com

ABSTRACT
Recent studies have focused on the impact of changes in thyroid hormone levels and its relation with the localization of IGF during healing process of periodontium .Thus, the aim of the present study was to evaluate the effects of thyrodoctomy on healing of periodontium histologically and immunohistochemicaly on IGF-1R. Twenty male New Zealand rabbits were randomly assigned to two groups, 10 healthy (control), and the other10 were subjected to subtotal thyroidectomy to induce hypothyroidism. Once alterations were confirmed by total serum levels of triiodothyronine and thyroxine after thyrodoctomy, ligatures were randomly placed around the first mandibular molars to induce periodontitis, the animals were sacrificed in two intervals 2 and 4weeks (10 rabbits for each) and specimens routinely processed for serial decalcified sections for histological and immunohistochemical study on IGF-1R. The histological results of the present study revealed delay in healing process of the periodontium of experimental group when compare with control one. Immunohistochemical findings shows higher immunoreactivity in the periodontium cells of hypothyroid group than control.Within the limits of the present study, a thyroid hormone-deficient state may cause delay healing of induce periodontitis with height reactivity to IGF-1R.

KEY WORDS: Thyrodoctomy, periodontitis, hypothyroidism, IGF-1R.

INTRODUCTION

Periodontitis are infections occur due to imbalance confront between bacterial and host response (Kornman *et al.*, 2000) The host reaction to microbial damage involves induction of inflammatory cells, and activation of osteoclasts, leading to alveolar bone resorption and detachement of PDL (Ishikawa, 2000). In vivo studies have demonstrated that systemic factors may also play an important role in periodontal disease initiation and progression (Genco, 1996). Triiodothyronine (T3) and thyroxine (T4) are hormones secreted by the thyroid gland, and have been shown to be essential for normal bone remodelling (Vestergaard and Mosekilde, 2002). In hypothyroidism, for instance, bone turnover is slow, bone growth and maturation are retarded in childhood and adults tend to exhibit osteosclerosis, accompanied by increased fracture risk (Little, 2006). IGFs represent a family of endocrine, paracrine, and autocrine-acting polypeptide growth factors controlling pre- and postnatal development and growth. The IGF ligands, IGF-I and -II are involved in various cellular activity, including differentiation, proliferation, morphogenesis, growth, and carcinogenesis. IGF-1 has been shown to be an influential regulator of endochondral ossification (Wang *et al.*, 2010). Previous studies in rodents and humans have reported that insulin-like growth factor (IGF) is associated with thyroid growth and goitrogenesis. Thyroid hormone may regulate IGF-1 signaling in growth plate cells predominantly at the receptor level (Ock *et al.*, 2011). IGF-1 has been described as a stabilizer of β-catenin, and thyroid hormone is a known stimulator of IGF-1 receptor expression (Wang *et al.*, 2010). The previous studies demonstrated that the IGF-1 system plays an important role in the biology of oral and facial tissues and organs, including the development and healing of the periodontium (Go¨tz, 2006).

MATERIALS & METHODS

Twenty New Zealand male rabbits, weighting (1.5– 2kg), aged (6-12) months were used in this study. The rabbits were randomly assigned to two groups, 10 healthy (control), and the other10 were subjected to subtotal thyroidectomy to induce hypothyroidism (Amadi *et al*, 2006). Once alterations were confirmed by total serum levels of triiodothyronine and thyroxine after thyrodoctomy using a commercial radioimmunoassay kit (Diagnostic products Ltd, Abingdon, UK). Ligatures were randomly placed around the first mandibular molar for all animals in both groups in order to induced periodontitis (Feitosa *et al.*, 2009). The animals were sacrificed in two intervals 2 and 4weeks (10 rabbits for each, 5 for control and 5 for experimental). All tissue specimens, experimental and controls were fixed in 10% neutral formalin and processed in a routine paraffin blocks after complete decalcification of bone. Each paraffin-embedded specimen from each block of all studied sample had serial sections were prepared in 4µm thickness and mounted on clean glass slides for routine H&E staining. Other 4µm thickness sections were mounted on positively

charged microscopic slides for immunohistochemical localization of IGF-1R .The procedure of the IHC assay was carried out in accordance with the manufacturer instructions of monoclonal Anti- antibody for IGF-1R (ab4065) Abcam UK and mouse specific HRP/DAB detection Kits System (ab 64259) Abcam UK.

RESULTS

Serum analysis results showed that there were highly significant differences between the levels of T3, T4 before and after thyroidectomy. Also there were highly significant increases in TSH level after surgery when compared with their levels before it (Table-1).

TABLE1: The levels of thyroid hormones (nmol/L) before and after surgery

Hormone	Before surgery	After surgery	T -value	Significant
T3	0.95±0.05	0.36±0.03	14.458	p≤0.0001
T4	61.16±2.54	29.99±2.1	12.234	p≤0.0001
TSH	0.331±0.04	0.983±0.1	7.83	p≤0.0001

Microphotograph view of control group at two weeks duration showed new collagen fibers almost filling the defect area with numerous blood vessel and new bone formation (Figures 1, 2). While the histological view of hypothyroidism group of the same periods revealed bone remodeling by presence of osteoclasts, osteoblasts and reversal line (Figure.3). Remodeling of collagen fibers with formative fibroblasts and osteoblasts also seen (Figure 4).

The histological view of six weeks duration of control group shows new bone formation with reattachment of periodontal fibers by presence of sharpy's fibers in bundle bone (Figure 5).The view of the experimental side revealed continuous bone remodeling by presence of osteoclasts, reversal line and remodelling of periodontal ligament by active fibroblasts and new collagen fibers (Figure 6).

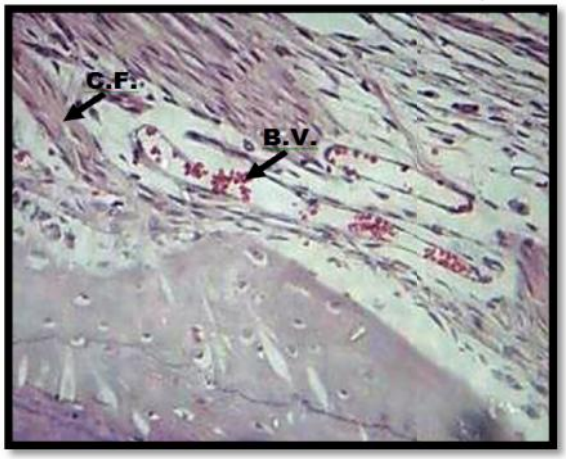


FIGURE 1: View of control group at 2 weeks shows new collagen fibers (C.F.), blood vessels (B.V.) and new bone formation. H&EX20.

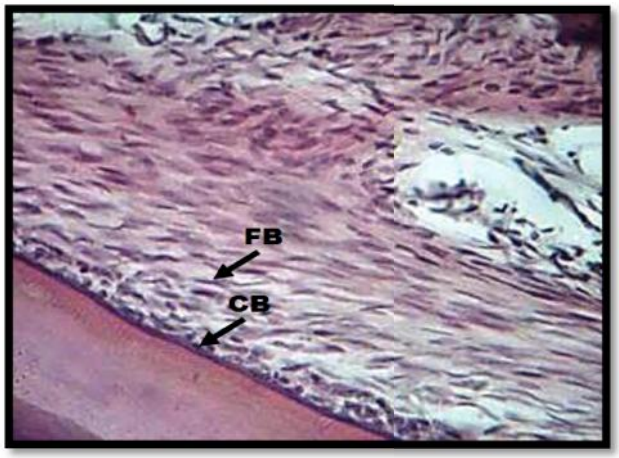
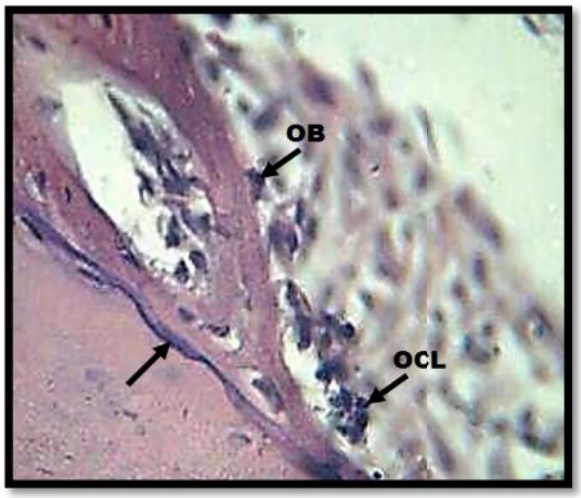


FIGURE 2: View of control group shows cementoblasts (CB) and fibroblasts (FB). H&E x20.



FIGURES 3: View of experimental group at 2 weeks shows new bone formation, osteoclasts (OCL), osteoblasts (OB), and reversal line (arrow). H&Ex100**.**

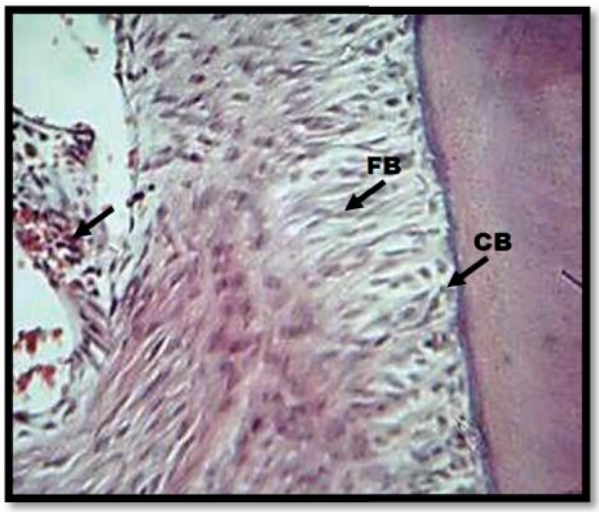


FIGURE 4: View of experimental group at 2 weeks duration shows new collagen formation, cementoblasts (CB), fibroblast (FB) and blood vessels (arrow). H&Ex40.

Immunohistochemical result:

Localization of IGF-R1 in control group at 2 weeks duration showed weak to moderate immunoreactivity appeared all over the extracellular matrix of the PDL, cementoblasts, osteoclasts and some osteoblasts, while the bone matrix and osteocytes did not react (Fig.7).

Regarding the experimental group, strong IGF-I immunostaining was detected in the PDL cells especially in fibroblasts and cementoblasts. Osteoclasts, osteocytes which were located near the periphery of bundle bone also positively stained (Fig.8).

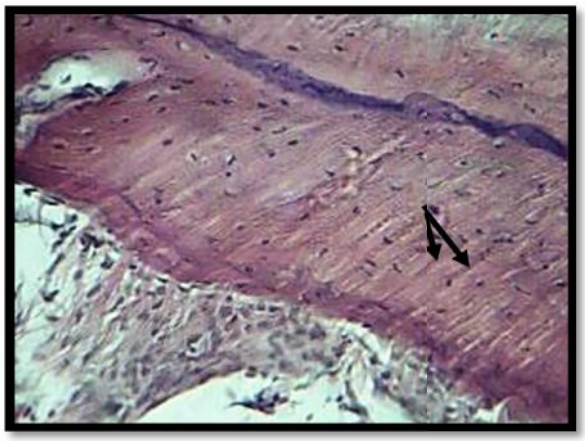


FIGURE 5: View of 6weeks control group shows sharpy's fibers (arrows) inside new bundle bone. H&Ex40.

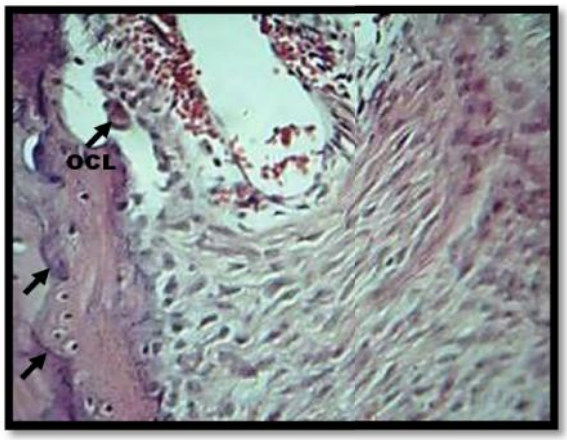


FIGURE 6: View of 6weeks experimental group shows bone remodeling with osteoclasts (OCL) and reversal line (arrows). H & Ex40**.**

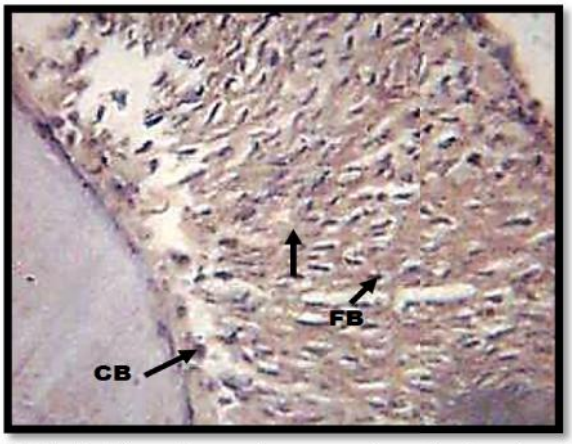


FIGURE 7: View of control group at 2 weeks duration shows positive expression of IGF-R1 by periodontal ligament fibers (arrows), Fibroblasts (FB) and cementoblasts (CB). DAB stain with counter stain hematoxylin X40.

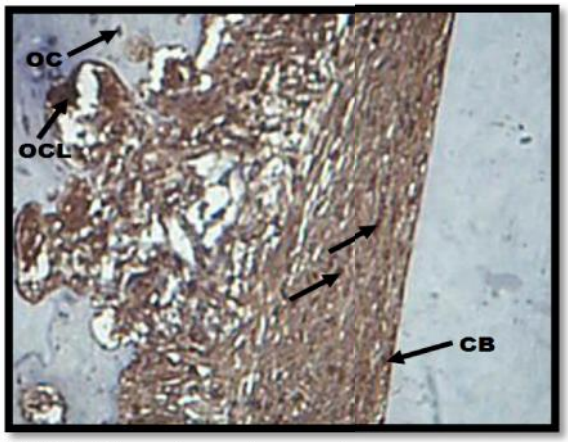


FIGURE 8: View of control group shows positive expression of IGF-R1 by osteocytes (Oc), osteoclasts (OCL), cementoblasts (Cb) and fibroblasts (arrows). DAB stain with counter stain hematoxylinX40.

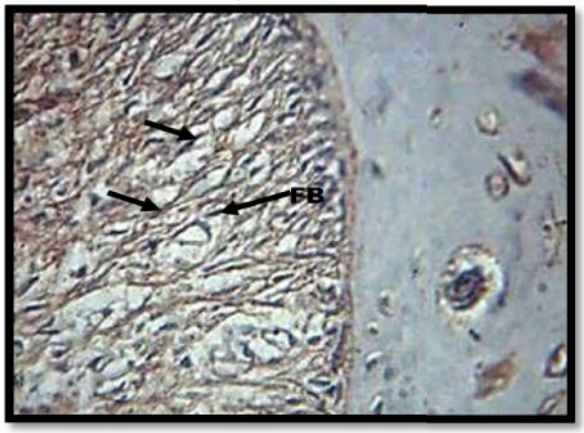


FIGURE 9: View of control group shows weak positive expression of IGF-R1 by fibroblasts (Fb) and periodontal ligament fibers (arrows). DAB stain with counter stain hematoxylinX40.

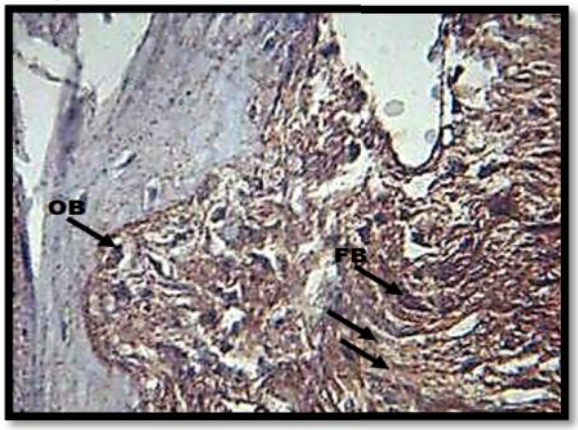


FIGURE 10: View of experimental group shows strong positive expression of IGF-R1 by osteoblasts (OB), Fibroblasts (FB) and periodontal ligament fibers (arrows). DAB stain with counter stain hematoxylin X40.

On 6 weeks, immunostaining in and around alveolar bone in control group began to decline, but fibroblasts and parts of the extra cellular matrix of periodontal ligament were still show weak IGF-I immunoreactivity (Figure 9). Whereas in experimental group there was still strong IGF-I immunoreactivity especially in osteoblasts, fbroblasts and in periodontal ligaments fibers (Figure 10).

DISCUSSION

In the present study, rabbits were subjected to sub-total thyroidectomy, the results shows decrease in T3, T4 and increase in TSH levels and this illustrate hypothyroidism (Yildirim *et al.,* 2008). This may be due to a decrease in the thyroid gland tissue, which cause reduction in the amount of T3 andT4 secreted into serum, as a result, an over secretion of TSH from the pituitary gland will occur to compensate this reduction (Watson *et al.,* 2009).

The histological findings of control group of 2weeks interval shows new bone formation with reattachment of periodontal fibers in both cementum and bundle bone ,whereas the periodontium healing picture in hypothyroid group revealed continuous remodeling in both periodontal ligament and bundle bone. This finding agree with previous studies (Williams, 2011) who said that his 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.

The histological picture of control animals at 4weeks duration manifested by mature bone, and osteocytes were trying to get concentric arrangement around haversian canal . While in hypothyroid group, the newly formed bone had immature bone with osteocytes which still irregularly arranged. Osteoclasts and reversal lines were widely seen in the newly formed bone which means continuous bone remodeling. This result correlate with previous study done by Williams in 2011, who found that when there is a deficiency in T3 or mutation in TRα (which is the receptor of T3in osteoblasts cells), there will be a delay in bone ossification and maturation.

Insulin-like growth factor-1 is involved in the formation, maturation and reparation processes of several tissues, when the tissues are experiencing a fast growth rate. All IGF-1 biological effects are mediated through interaction with its specific cell surface receptor, which is responsible of mitogenic signaling and most of the effects that IGF-1 promotes during tissue growth, development and repair (D'Ercole, 1996). It has been demonstrated that IGF-1 regulates cell growth and differentiation in developing tissues and modulates some functions in mature cells (Joseph *et al*, 1993). This could explain IGF-1receptors showed immunohistochemical staining in some of the periodontium cells of both groups in this study.

The result of this study revealed that the expression of IGF-1R monoclonal antibodies was more evident in hypothyroid groups by various cellular and fibrillar elements of the periodontium including osteoblasts, cementoblasts, fibroblasts and PDL fibers throughout healing intervals. A number of previous studies have shown that insulin receptors are expressed in osteoblast-like cells and have documented specific actions including effects on collagen synthesis and alkaline phosphatase formation (Al-Talabany, 2012). Osteoclasts were also immunoreactive for IGF-1R, this is in accordance with findings for osteoclasts from different species (Go¨tz, 2006) It remains unclear whether these increased immunoreactivities are the result of increased synthesis of IGF components or an increased accumulation from the circulation (Go¨tz, 2006) Cementoblasts were immunoreactive for IGF-1R.This finding indicates that the IGF system, besides other growth factor families, plays a role in the repair process.

A compensatory increase of IGF-1R expression, suggesting a major role for the IGF-1R in the regulation of thyroid function or growth (Ock *et al.*, 2011). As IGF-I regulates osteoprotegerin (OPG) and receptor activator of nuclear factor B ligand (RANKL), thus influencing bone resorption as well as formation activity (Ueland, 2005).

Specific functions of the IGFs may be localized in particular tissue compartments. In the cementum, several IGF components were found indicating roles in tissue homeostasis or attachment. The PDL may function as a reservoir for IGFs probably bound to ECM components. PDL fibroblasts could then respond in a paracrine manner (Go¨tz, 2006)

Thus, the decrease in the levels of thyroid hormones may promote a less expert immunogenic response to the infection induced by the experimental periodontitis (Bucay, 2007) and, therefore, it may be suggested that the role of hypothyroid on the progression of periodontitis may be related to a relationship between the immune system and the thyroid axis, and not related to the effect of thyroid hormone changes on the alveolar bone quality (Klecha *et al.*, 2000).

CONCLUSION

The present study concluded that a thyroid hormone-deficient state may cause delay healing of induce periodontitis when compare with control one with height positive reactivity of periodontium cells of hypothyroid group to IGF-1R than control one.

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