CAN THYROID DYSFUNCTION INDUCE PERIODONTAL DISEASE?

Adriana Monea, Lecturer, DMD, PhD
Department of Odontology and Periodontology
Faculty of Dental Medicine, UMF Tirgu-Mures, Romania
Nagy Elod, Associate Professor, MD PhD
Department of Pharmaceutical Biochemistry
Faculty of Pharmacy, UMF Tirgu-Mures, Romania
Alexandru Sitaru, PhD Student
Department of Odontology and Periodontology
Faculty of Dental Medicine, UMF Tirgu-Mures, Romania
Alexandra Stoica, Resident
Department of Oodntology and Periodontology
Faculty of Dental Medicine, UMF Tirgu-Mures, Romania
Monica Monea, Associate Prof., DMD, PhD
Department of Odontology and Periodontology
Faculty of Dental Medicine, UMF Tirgu-Mures, Romania

Abstract

Objective: to determine if thyroid dysfunction can induce periodontal disease, by comparing salivary and serum levels of IL-6 and TNF-α in patients with thyroid dysfunction without clinical periodontal changes to healthy controls.

Material and Methods: 28 randomly selected adult patients with thyroid dysfunction and 24 healthy adults were enrolled. Venous blood and un-stimulated saliva were assessed for TNF-α and Interleukin-6 levels, using ELISA-sandwich methods. Comparisons between groups were made using Mann-Whitney and Kruskal-Wallis tests, and the level of significance was set at $p \leq 0.05$.

Results: Mean values of serum TNF-α concentration for patients with hypothyroidism (132.29±91.26pg/ml) were 4 times higher than in subjects with hyperthyroidism (32.24±29.46pg/ml) and almost 15 times higher than in controls, but there was no significant difference between hypo- and hyperthyroid subjects. Mean values of salivary TNF-α were significantly higher in hyperthyroid subjects than in controls ($p=0.011$), also in hypothyroid subjects than in controls ($p=0.009$), but there was no significant

difference between hypo- and hyperthyroid subjects. Serum IL-6 had apparently much higher levels in study groups (99.39±54.41pg/ml hyperthyroid and 68.69±45.94pg/ml in hypothyroid) compared with controls (11.23±2.14pg/ml), but the differences were not statistically significant. Mean values of salivary IL-6 were significantly higher both in hyperthyroid and hypothyroid subjects than in controls ($p<0.001$), and there was an extremely significant difference between hypo- and hyperthyroid subjects ($p<0.001$).

Conclusion: Serum and salivary levels of TNF-α and IL-6 represent a well individualized biologic indicator for appreciating the development of periodontitis in subjects with thyroid dysfunction. Hyperthyroidism can induced more important periodontal destructions than hypothyroidism.

Keywords: Thyroid dysfunction, TNF-alpha, IL-6, periodontal status

Introduction

Triiodothyronine (T3) and thyroxine (T4) were demonstrated to have a fundamental role in normal growth, development, skeletal maturation and bone turnover (Ganong 2001, Bland 2000). Variations in blood levels of T3 and T4 are strongly associated with hypo- and hyperthyroidism, with clinical consequences upon bone growth and development (Little 2006). Thus, in hypothyroidism the bone turnover is slow, bone growth and maturation are retarded and these patients will exhibit in time osteosclerosis and high risk of bone fracture (Allain et al 1995, Vestergaard 2002). In hyperthyroidism there is accelerated bone maturation with reduced bone mineral density, high bone turnover and a negative calcium balance (Allain et al 1995, Vestergaard 2002, Suwanwalaikorn et al 1996, Siddiqi et al 1998). Alveolar bone resorption is the most important clinical parameter used to assess the severity of periodontal disease; therefore, variations of T3 and T4 blood levels may be considered a modulating factor in chronic marginal periodontitis.

Several studies showed that thyroid hormones play an important role in bone resorption by influencing osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL) mechanism (Akalin et al 2002). This is accompanied by local production of IL-6 and other bone regulating factors (Siddiqi et al 1998, Siddiqi et al, 1999, Tokuda et al, 1998). Thyroid dysfunction has a negative effect on IL-6 and TNF-alpha, which play an important role in osteoclast differentiation and function, without any influence from RANKL-RANK interactions (Kanatani et al 2004).

The aim of this research was to compare the salivary and serum levels of IL-6 and TNF-α in patient with thyroid dysfunction without periodontal

changes to healthy controls and to identify whether these cytokines might be indicators for the development of periodontal disease.

Material and methods

Subjects:

28 randomly selected patients (19 female and 9 male, age range: 23-53 years) with thyroid dysfunction were enrolled in the study. Thyroid dysfunction was diagnosed by the endocrinologist based on clinical signs and lab examinations, such as TSH, T3 and fT4 serum levels, and all subjects were under specific treatment for their thyroid dysfunction at the time of enrollment.

These subjects were divided according to their thyroid dysfunction into 2 groups: hyperthyroidism (n=15, 10 female and 5 male), and hypothyroidism (n=13, 9 female and 4 male).

24 volunteers (19 women and 5 men) of good general health condition and no thyroid dysfunction, no ongoing disease and no medication, served as controls.

Inclusion criteria were: more than 18 years of age, presence of at least 20 sites per jaw, no periodontal problems or periodontal treatment within the last six months and no medication intake that might have an influence on periodontal status or might have been prescribed for long-term treatment of metabolic diseases such as rheumatoid arthritis or osteoporosis.

Smokers, subjects over 60 years of age, or patients presenting any kind of inflammatory disease within the last 3 months were excluded from the study.

The study protocol had been approved by the local Ethical Committee (Number 16/18.07.2011) of the University of Medicine and Pharmacy from Tirgu-Mures. All patients were asked to sign an informed consent.

Method of analysis:

After obtaining informed consent, 2 ml of un-stimulated saliva were collected into a sterile tube from each enrolled subject, centrifuged for 3 min at 3.000 rpm and clarified supernatant was filtered through a low protein binding membrane, separated in polyethylene recipients, then marked for identification and stored in the refrigerator until examination.

Venous blood samples were obtained by venipuncture using an adequate closed system sample. Tubes were then centrifuged at 3,000 rpm/10 min and stored at -20^{o}C until analyzed.

Salivary and serum TNF-α levels were assessed with the ELISA-sandwich method using a commercially available immunoassay kit (OptEIA human TNF-α, Pharmingen, USA) according to the manufacturer's

guidelines. The values of salivary and serum TNF-α levels were extrapolated from a curve drawn using TNF-α standard and reported in pg/ml.

Saliva and serum interleukin-6 levels were evaluated using a commercially available enzyme-linked immunoabsorbent, sandwich ELISA assay (DuoSet ELISA Development System, R&D Systems, USA) according to the manufacturer's guidelines. Results were reported as total amount of IL-6 (in pg/ml).

Statistical Analysis:

Statistical analysis was performed using Microsoft Excel 2003 and Statistica 5.0. Data are expressed as mean and standard deviation (SD). Kolmogorov-Smirnov and Lilliefors tests were used to analyze statistic distribution of parameters. Parametric and nonparametric tests were used to compare variables. Comparisons between groups were made for non-normally distributed variables using Mann-Whitney U and Kruskal-Wallis tests. The level of significance was set at $p \leq 0.05$.

Results. The mean age for the subjects with hyperthyroidism was 43.2±2.94 years and 46.15±2.36 years for patients with hypothyroidism. The healthy controls were aged 42.12±3.34. The groups did not differ significantly from each other with regards to the mean age of the subjects. General clinical parameters (mean ± SD) of the 2 groups with thyroid dysfunction are provided in Table 1. The Body Mass Index (BMI) of the patients with hypothyroidism was significantly increased compared to patients with hyperthyroidism and controls. The serum glucose level did not vary among groups, whereas TSH levels displayed a statistically significant increase in patients with decreased thyroid function and FT_4 displayed a statistically significant increase in patients with hyperthyroid function, respectively. Healthy controls had all the clinical parameters at normal values. All subjects in the study groups presented healthy periodontal tissues.

Table 1. Clinical parameters in patients with thyroid dysfunction

Hyperthyroidism	**Nr. of cases**	**Mean value**	**Minimum**	**Maximum**	**Standard deviation**
BMI	15	21.23	12.89	32.38	2.30
Glycemia (mg/dL)	15	90.88	71	106	4.60
TSH (uUI/mL)	15	0.069	0.005	0.23	0.09
FT4 (ng/dL)	15	2.86	0.47	13.62	0.82
Hypothyroidism					
BMI	13	28.29	22.83	33	2.09
Glycemia (mg/dL)	13	95.63	65	118	4.37
TSH (uUI/mL)	13	17.64	1.52	77.44	7.78
FT4 (ng/dL)	13	0.95	0.16	1.58	0.14

In 50 out of 52 subjects, serum TNF-α was detected. Serum TNF-α concentrations were 32.24±29.46 pg/ml in subjects with hyperthyroidism and 132.29±91.26 pg/ml in subjects with hypothyroid function, respectively. Mean values of serum TNF-α of patients with hypothyroid function showed an approximately 4 times higher level than in hyperthyroid subjects and almost 15 times higher level than in controls. However, there was no significant difference between hypo- and hyperthyroid subjects (Table 2). Serum TNF-α showed a positive correlation with age in hyperthyroid subjects with tendency to statistic signification (r=0.44, p=0.11).

Table 2. Variation of serum TNF-α in study groups and comparisons with controls

Groups	Serum TNF-α (pg-mL) mean. ± std. err.
Hyperthyroidism (n=14)	32.24 ± 29.46*
Hypothyroidism (n=12)	132.29 ± 91.26†
Controls (n=24)	8.69 ± 1.76

p=0.05, Kruskal-Wallis ANOVA test
**p>0.05, †p<0.05,‡p<0.01, §<0.001*
All groups were compared with the control group

Increased levels of serum TNF-α in hypothyroid subjects can have two explanations: BMI in subjects with hypothyroid function was increased (mean value ± std. err. 28.29±2.09), showing first degree obesity; on the other hand, three patients with hypothyroidism had anti-TPO auto antibodies (autoimmune thyroiditis), that can be the reason for the increased cytokine levels. Two of these subjects had TNF-α value of 966 pg/mL, respectively 613 pg/mL.

Salivary TNF-α was detected in 50 of 52 samples. Hyperthyroid subjects expressed a higher level of TNF- α in the saliva than hypothyroid, and both groups had higher salivary TNF-α levels than controls (p<0.05) (Table 3).

Table 3. Measured salivary TNF- α and comparisons with controls

Groups	Salivary TNF-α (pg-mL) mean. ± std. err.
Hyperthyroidism (n=14)	11.52 ± 3.08†
Hypothyroidism (n=12)	10.53 ± 3.24‡
Controls (n=24)	8.86 ± 4.68

p=0.05, Kruskal-Wallis ANOVA test
**p>0.05, †p<0.05,‡p<0.01, §<0.001*
All groups were compared with the control group

There was no correlation of salivary TNF-α with any other clinical parameter, irrespective of the thyroid dysfunction, but we found a significant positive correlation between salivary TNF-α and salivary IL-6 in the total study group (r=0.410, p=0.006).

Serum IL-6 had apparently much higher levels in study groups (99.39±54.41 pg/ml hyperthyroidism and 68.69±45.94 pg/ml in hypothyroidism) compared to controls (11.23±2.14 pg/ml), but the differences were not statistically significant (Table 4).

Table 4. Serum IL-6 variation in study groups and comparisons with controls

Groups	Serum IL-6 (pg-mL) mean. ± std. err.
Hyperthyroidism (n=15)	99.39 ± 54.41*
Hypothyroidism (n=13)	68.69 ± 45.94*
Controls (n=24)	11.23 ± 2.14

p=0.05, Kruskal-Wallis ANOVA test

**p>0.05, †p<0.05,‡p<0.01, §<0.001*

All groups were compared with the control group

Salivary IL-6 levels were significantly higher in hyperthyroid subjects than in hypothyroid ones, and both thyroid dysfunction groups had significant higher levels than healthy controls (p<0.001) (Table 5).

Table 5. Measured salivary IL-6 and comparisons with controls

Groups	Salivary IL-6 (pg/mL)mean. ± std. err.
Hyperthyroidism (n=15)	21.07 ± 6.71§
Hypothyroidism (n=13)	16.63 ±2.36§
Controls (n=24)	7.64 ± 2.98

p=0.05, Kruskal-Wallis ANOVA test

**p>0.05, †p<0.05,‡p<0.01, §<0.001*

All groups were compared with the control group

Discussions. Interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) are two major pro-inflammatory cytokines that are locally produced in different tissues in different pathological situations, including thyroid dysfunction (Bartalena et al 1994, Sekeroglu et al2006). These locally produced factors enter systemic circulation and spread in whole body, including into periodontal tissues. It is thought that cytokines stimulate resident cells of the periodontium to produce metalloproteinases, molecules that mediate connective tissue destruction and induce the differentiation and activation of osteoclasts, leading to alveolar bone destruction (McGee et al, 1998).

In our study, the cytokines that were produced due to thyroid dysfunction might have been the initiators of an amplified inflammatory cascade. In combination with endotoxins produced by germs in dental plaque this might lead to higher local inflammatory mediator concentrations, including cytokines and prostaglandins, higher concentrations of matrix metalloproteinase (MMP) and of other proteinases with destructive effects on bone and conjunctive tissue, in the end leading to osteoporosis and periodontal breakdown.

Recently, several studies were published regarding the correlation between thyroid dysfunction and inflammatory markers. Sekeroglu et al. (2006) suggested that in the absence of non-thyroidal dysfunction, IL-6 might be considered a useful marker for thyroid destructive processes, with a direct augmentative effect on cytokine serum concentrations. Furthermore, it was suggested that IL-6 production from mononuclear cells in hyperthyroid women was higher than in controls (Lakatos et al 1997). There are several mechanisms which can lead to increased levels of serum IL-6, a common feature of hyperthyroidism. Among these are: bone resorption, autoimmune inflammatory conditions or excessive concentrations of T3 and T4, all induced by thyrotoxicosis (Fenkci et al 2001). Regardless of the mechanisms involved, in hyperthyroidism there will always be an increased level of IL-6, which in turn will have a stimulatory effect on osteoclasts differentiation and proliferation, with bone resorption as final effect.

In bone turnover there are two important metabolic mechanisms: new bone formation by osteoblasts and old bone degradation by osteoclasts. Total bone mass is influenced by the balance between bone formation and resorption. Assessment of bone formation markers (osteocalcin and alkaline phosphatase) in hyper- and hypothyroid patients pointed to the fact that bone turnover was much higher in hyperthyroid subjects. But by determining the serum IL-6, IL-8 and TNF-α levels there was no correlation found between cytokines and bone turnover markers (Sekeroglu et al 2006). Celik et al. (1995), however, suggested that resorptive effect on the bone in hyperthyroidism is supposed to be cytokine mediated. Chopra et al. (1991) reported no significant difference between serum TNF-α level in hypothyroid and hyperthyroid subjects vs. controls, whereas Siddiqi et al. (1999) reported higher serum TNF-α levels in hyperthyroid patients. This is in contrast to our findings. In the present study, hypothyroid patients had an increased Body Mass Index (BMI) pointing to an obesity tendency and causing higher serum levels of these cytokines (Vikram 2011). Additionally, three of the hypothyroid patients had anti-TPO antibodies (autoimmune thyroiditis). These findings might explain the high serum TNF-α level in patients with a decreased thyroid function.

On the other hand, several clinical studies demonstrated that periodontal disease itself determines higher serum and salivary pro-inflammatory cytokines levels, especially TNF-α, IL-10 and IL-6 (Ng et al 2007, de Queiroz et al 2008, Frodge et al 2008, Lia Coman 2009). Our results showed higher levels than those of de Queiroz and coworkers (2008). These authors reported serum TNF-α levels of 6.4±17.3 pg/ml in 17 subjects with chronic periodontitis compared with serum TNF-α levels of 0.9±1.8 pg/ml in 8 healthy controls. In 2009, Coman (Lia Coman 2009) described serum TNF-α levels of 9.8±2.35 pg/ml in subjects with periodontal disease.

The significant differences might be due to systemic condition, confirming the hypothesis that systemic disease exacerbates periodontal disease.

Likewise, the salivary cytokine levels determined were higher than those described by different authors who investigated subjects with periodontal disease but systemically healthy. In our study we measured salivary TNF-α levels of 11.52±3.08 pg/ml and salivary IL-6 of 21.07±6.71 pg/ml in subjects with hyperthyroidism, respectively salivary TNF-α levels of 10.53±3.25 pg/ml and salivary IL-6 of 16.63±2.36 pg/ml in subjects with hypothyroidism. Ng et al. (2007) reported salivary TNF-α levels of 1.9±0.2 pg/ml and salivary IL-6 of 15.1±47.8pg/ml in 98 subjects with periodontal disease. In 2008, in 35 subjects with moderate and severe periodontitis salivary TNF-α levels of 4.33 pg/ml were reported (Frodge et al 2008), and in 2009 salivary TNF-α levels of 7.31±0.91 pg/ml were described in periodontal disease subjects (Lia Coman 2009). The significant differences salivary TNF-α levels are due to systemic disease, such as thyroid dysfunction.

Our results point to the fact that thyroid hormones, in lower or higher concentrations than normal, might co-induce periodontal disease, by raising serum and salivary cytokine levels, which activate different pathways determining alveolar bone and conjunctive tissue destruction.

Despite of very significantly increased concentrations of cytokines, no correlations between cytokine levels and periodontal status was found, since the subjects included in this study had no clinical modifications of periodontal tissues yet. Similar results were reported by Scapoli et al. (2007) and Teles et al. (2009).

Conclusion

In conclusion, the present results suggest that serum and salivary levels of pro-inflammatory cytokines TNF-α and IL-6 represent a well individualized biologic indicator for assessing the possible development of periodontal disease in subjects with thyroid dysfunction. Hyperthyroidism can induce more important periodontal destructions than hypothyroidism, with both thyroid dysfunctions having an effect on periodontal tissues through inflammatory mediators such as cytokines. Determining TNF-α and IL-6 levels in total saliva is a comfortable, simple and relatively safe method for diagnosing the influence of some systemic conditions on periodontal tissues.

References:

Akalin A, Colak Ö, Alatas Ö , Efe B. Bone remodeling markers and serum cytokines in patients with hyperthyroidism. Clinical Endocrinology 2002; 57:125–129.

Allain TJ, Thomas MR, McGregor M, Salisbury R. A histomorphometric study of bone changes in thyroid dysfunction in rats. Bone 1995; 16:505–509.

Bartalena L, Grasso L, Brogioni S, Aghini-Lombardi F, Braverman LE, Martino E. Serum interleukin-6 in amiodarone-induced thyrotoxicosis. Journal of Clinical Endocrinology & Metabolism 1994; 78:423-427.

Bland R. Steroid hormone receptor expression and action in bone. Clinical Science 2000; 98:217–240.

Celik I, Akalin S, Erbas T. Serum levels of interleukin 6 and tumor necrosis factor-alpha in hyperthyroid patients before and after propylthiouracil treatment. European Journal of Endocrinology 1995; 132:668-672.

Chopra IJ, Sakane S, Teco GN. A study of the serum concentration of tumor necrosis factor alpha in thyroidal and non-thyroidal illnesses. Journal of Clinical Endocrinology & Metabolism 1991; 72:1113-1116.

de Queiroz AC, Taba M Jr, O'Connell PA, da Nóbrega PB, Costa PP, Kawata VK, Trevisan GL, Novaes AB Jr, de Souza SL, Palioto DB, Grisi MF. Inflammation markers in healthy and periodontitis patients: a preliminary data screening. Brazilian Dental Journal 2008: 19:3-8.

Fenkci S, Corapcioglu D, Erdogan G. The effects of thyrotoxicosis on serum IL-6 and bone turnover in premenopausal women. Turkish Journal of Endocrinology & Metabolism 2001; 3:97-101.

Frodge BD, Ebersole JK, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. Journal of Periodontology 2008; 79:1913-1919.

Ganong WF. Review of Medical Physiology. 20th ed. New York, NY: McGraw-Hill 2001; 307-321.

Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. Journal of Cell Physiology 2004; 201:17–25.

Lakatos P, Foldes J, Horvath C, Kiss L, Tatrai A, Takacs I, Tarjan G, Stern PH. Serum interleukin-6 and bone metabolism in patients with thyroid function disorders. Journal of Clinical Endocrinology & Metabolism 1997; 82:78-81.

Lia Coman M. Immunological research in periodontal disease. PhD Thesis 2009; UMF Tg.Mures

Little JW. Thyroid disorders. Part I: hyperthyroidism. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics 2006; 101:276–284.

McGee JM, Tucci MA, Edmundson TP, Serio CL, Johnson RB. The relationship between concentrations of proinflammatory cytokines within

gingiva and the adjacent sulcular depth. Journal of Periodontology 1998; 69:865-871.
Ng PY, Donley M, Hausmann E, Hutson AD, Rossomando EF, Scannapieco FA. Candidate salivary biomarkers associated with alveolar bone loss: cross-sectional and in vitro studies. FEMS Immunology & Medical Microbiology 2007; 49:252-260.
Scapoli C, Mamolini E, Trombelli L. Role of IL-6, TNF-A and LT-A variants in the modulation of the clinical expression of plaque-induced gingivitis. Journal of Clinical Periodontology 2007; 34:1031–1038
Sekeroglu MR, Altun ZB, Algün E, Dülger H, Noyan T, Balaharoglu R, Oztürk M. Serum Cytokines and Bone Metabolism in Patientes with Thyroid Dysfunction. Advances in Therapy 2006; 23:475-480.
Siddiqi A, Burrin JM, Wood DF, Monson JP. Tri-iodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. Journal of Endocrinology 1998; 157:453-461.
Siddiqi A, Monson JP, Wood DF, Besser GM, Burrin JM. Serum cytokines in thyrotoxicosis. Journal of Clinical Endocrinology & Metabolism 1999; 84:435-439.
Suwanwalaikorn S, Ongphiphadanakul B, Braverman LE, Baran DT. Differential responses of femoral and vertebral bones to long-term excessive L-thyroxine administration in adult rats. European Journal of Endocrinology 1996; 134:655–659.
Teles RP, Likhari V, Socransky SS, Haffajee AD. Salivary Cytokine Levels in Chronic Periodontitis and Periodontally Healthy Subjects. A crosssectional Study. Journal of Periodontal Research 2009; 44:411–417.
Tokuda H, Kozawa O, Harada A, Isobe KI, Uematsu T. Triiodothyronine modulates interleukin-6 synthesis in osteoblasts: inhibitions in protein kinase A and C pathways. Endocrinology 1998; 139:1300–1305.
Vestergaard P, Mosekilde L. Fractures in patients with hyperthyroidism and hypothyroidism: a nationwide follow-up study in 16,249 patients. Thyroid 2002; 12: 411–419.
Vikram NK, Bhatt SP, Bhushan B, Luthra K, Misra A, Poddar PK, Pandey RM, Guleria R. Associations of -308G/A polymorphism of tumor necrosis factor (TNF)-α gene and serum TNF-α levels with measures of obesity, intra-abdominal and subcutaneous abdominal fat, subclinical inflammation and insulin resistance in Asian Indians in north India. Disease Markers 2011; 31(1):39-46.