INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 6, p. 122-128, 2018

RESEARCH PAPER OPEN ACCESS

Serum and salivary levels of thyroid antibodies (TPO-Ab&Tg-Ab) in the of hypothyroid patients with and without periodontitis

Sahar H. Al-Hindawi*, Batool H. Al-Ghurabi

Department of Basic Science, College of Dentistry, University of Baghdad, Iraq

Key words: *Hypothyroidism,* autoantibodies, *periodontitis, saliva, TPO-Ab, Tg-Ab.*

http://dx.doi.org/10.12692/ijb/13.6.122-128 Article published on December 23, 2018

Abstract

Hypothyroidism is the decrease in thyroid hormones production and thyroid gland function. Hashimoto's thyroiditis is the most common cause of hypothyroidism with production of autoantibodies directed toward autoantigens thyroglobulin (Tg) and thyroid peroxidase (TPO). This study was carried out to determine and compare serum and salivary levels of thyroid antibodies (TPO-Ab and Tg- Ab) in hypothyroid patients (with and without periodontitis) and healthy control; as well as to estimate the possibility to evaluate and measured these antibodies in the saliva as measured in the serum. Serum and saliva samples were collected from sixty hypothyroid patients with age ranged (20-64) years (30 of patients were with periodontitis and 30 without periodontitis), compare with 30 subjects as control with age ranged (20-53) years. Enzyme linked immunosorbent assay (ELISA) was used to detect of TPO-Ab and Tg-Ab in the serum and saliva. The results showed elevated serum levels of TPO-Ab and Tg-Ab significantly ($P<0.05$) in patients groups as compared to control, whereas there are non-significant differences ($P>0.05$) between two patients groups (with and without periodontitis). However, there are non-significant differences ($P>0.05$) in salivary levels of TPO-Ab and Tg-Ab between two patients groups as compared with control; as well no significant differences were found between two patient groups. The current findings suggest that the presences of thyroid antibodies TPO-Ab and Tg-Ab which may contribute in the pathogenesis of hypothyroidism in autoimmune state. However, salivary concentration of these antibodies cannot reflect the concentration the in serum.

* **Corresponding Author:** Sahar H. Al-Hindawi ✉ saharhashim10@yahoo.com

Introduction

Hypothyroidism is defined by a decrease in thyroid hormones production and thyroid gland function (Wiersinga, 2016). Hashimoto's Thyroiditis (HT) is the most common type of thyroiditis and the most common cause of hypothyroidism (DeRuiter, 2002).In HT, there is a lymphocytic infiltrate into the gland and production of autoantibodies directed toward autoantigens thyroglobulin (Tg) and thyroid peroxidase (TPO) (Wiersinga, 2016).

Thyroid peroxidase (TPO) is the key thyroid enzyme catalyzing both the iodination and coupling reaction for the synthesis of thyroid hormone. It is membrane bound and found in the cytoplasm and in high concentration (Swain *et al.,* 2005). Anti-TPO autoantibodies are found in over 90% of patients with autoimmune hypothyroidism and Graves' disease. Together with TG antibodies these are the predominant antibodies in autoimmune hypothyroidism (Silva *et al.,* 2003). Tg is secreted by the thyroid follicular cells into the follicular lumen and stored as colloid. Each Tg molecule has around 100 tyrosine residues; these residues couple to form the thyroid hormones triiodothyronine (T3) and thyroxin (T4). When TSH stimulates the thyroid cell, Tg is endocytosed and hydrolyzed in lysosome releasing T3 and T4. Tg- autoantibodies are found in less than 60% of patients with lymphocytic thyroiditis and 30% of Graves' disease patients (Swain *et al.,* 2005).

Periodontitis is the most common form of dental disease, comprise a number of inflammatory and infectious conditions caused by the inflammatory host response to bacteria in the gingival biofilm resulting in progressive destruction of periodontal ligaments and alveolar bone with pocket formation, recession or both (Pushparani, 2015; Gomes *et al.,* 2016). It is a chronic inflammatory disease that results in the breakdown of tooth-supporting tissues, ultimately leading to resorption of the alveolar bone (Pushparani, 2015).

The alterations in the hormonal regulation system have a substantial impact on periodontal tissues, by direct changes such as soft tissue edema, demineralization and pathological growth of the alveolar bone, or indirect changes through enzymes' defects which all modify the response of periodontal tissues to microbial pathogens (Zahid *et al.,* 2011).

Saliva is an important biological fluid in oral physiology (Holsinger and Bui, 2007), and a major player in the process of oral and general health maintenance (Humphrey and Williamson, 2001). According to several data it mirrors general health condition thus reflecting various systemic changes in the body (Chiappelli *et al.,* 2006; Nagler, 2008). There has been increasing interest in diagnosis based on saliva analyses (Javaid *et al*., 2016).

This study was carried out to determine and compare serum and salivary levels of thyroid antibodies (TPO-Ab and Tg- Ab) in hypothyroid patients (with and without periodontitis) and healthy control; as well as to estimate the possibility to evaluate and measured these antibodies in the saliva as measured in the serum.

Subjects and methods

Subjects

This study was enrolled 60 (4 males and 56 females) Iraqi hypothyroid patients their age ranged (20- 64) years, were rounded up from Nuclear Medicine and Radiation Therapy Department, Educational Oncology Hospital. The diagnosis of hypothyroidism was based on the clinical features and biochemical tests that depended mainly on elevated serum levels of TSH, low T_4 level, and low or normal T_3 as compared with control. All the patients had no complain of other chronic or systemic diseases, and pregnant women were excluded from the study. Total patients divided into two groups; 30 patients without periodontitis and 30 patients were diagnosed with periodontitis [the diagnosis was made through specialized dentists in the department of periodontics, College of Dentistry, Baghdad University]. Beside 30 (3 males and 27 females) volunteers subjects who were considered as control,

their ages and gender were matched with patients, their age ranged between (20-55) years.

Sample collection

Serum and saliva samples were collected from study groups. Approximately (4 ml) of human blood was collected intravenous from patient and control groups; also, un-stimulated saliva (3 ml) was collected from studies groups. Both of them centrifuged; then serum of blood was immediately separated and the supernatant of saliva aspirated immediately, both divided into aliquots and kept at -20 °C until used.

Detection of Antibodies

Detection of TPO-Ab and Tg-Ab level in the saliva were determined by using commercially available kits, Anti TPO-Ab ELISA Kit and anti TG-Ab ELISA Kit, from (MyBiosource, USA). Detection of TPO-Ab and Tg-Ab level in the serum were determined by using commercially available kits, Anti TPO-Ab ELISA Kit and anti TG-Ab ELISA Kit, from (Demeditec, Germany).

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to identify effect of difference factors in study parameters. The outcome quantitative variables were normally distributed, and therefore conveniently described by mean, standard error (SE) and tested for statistical significance by t-test and ANOVA test with least significant difference (LSD).

Results and discussion

Demographic Characteristics

The age of hypothyroid patients ranged between (20-64) years with a mean age of (39.88 ± 1.423) years; while the age of control group ranged between(20-53) with mean age (42.23 ± 1.657) years. However, the majority (43.33%) of patients are in the age group of (35-45) years. Furthermore, there is a significant female's predominance (93.33%) among patients group, as shown in table (4-1). Males/females ratio was (1:14), and no statistically significant differences (p>0.05) in age or gender existed between patients and controls, as shown in table (1).

Table 1. Distribution of studied groups by age and gender.

		Patients group		Controls group		t-test
Age	Range	20 - 64 years		20 - 53 years		$P>0.05^{NS}$
	Mean ± SE	39.88 ± 1.423		42.23 ± 1.657		
		P=0.782 t=0.124				
Age group		No.	%	No.	%	
	< 34	16	26.67	8	26.67	$P>0.05^{NS}$
	35- 45	26	43.33	10	33.33	
	> 46	18	30.00	12	40.00	
	Total	60	100%	30	100%	
	*Chi-square=4.025; P=0.008***					
Gender	Male	4	6.67	3	10	$P>0.05^{NS}$
	Female	56	93.33	27	90	
	Total	60	100%	30	100%	
	*Chi-square=1.89; P<0.001***					

SE: Standard Error; NS: No Significant; **: Highly Significant (P<0.001).

These results are consistent with other Iraqi study reported by Al-Shaibani *et al.* (2014), who demonstrated that the mean age of Iraqi hypothyroid patients was (39.9±14.23) years, and high prevalence of hypothyroidism in patients is in age groups were (30-39) and (40-49) years. While, the mean age of hypothyroid patients in other study conducted by Ajeena (2013)was (32.05±6.45), which is little low than that of the present result. Moreover, the current results are compatible with broad studies of Vanderpump and Tunbridge (2002) and Saha *et al.* (2007), who showed that hypothyroidism was more prevalent (40.5%) in the age group (36-45) years and the age preponderance of 34 years and above.

Table 2. Serum and salivary TPO-Ab and Tg-Ab levels in study groups.

Thyroid Ab. (Mean ± SE)	Serum TPO-Ab (IU/ml)	Serum Tg-A (IU/ml)	Saliva TPO-Ab (ng/ml)	Saliva Tg-A (U/ml)
Hypothyroidism	90.55 ± 20.42 A	132.84 ± 41.56 A	0.703 ± 0.12	2.70 ± 0.24
Hypo. with Periodontitis	121.09 ±26.96 A	131.16 ± 41.16 A	0.779 ± 0.21	3.55 ± 0.67
Control	35.42 ± 8.42 B	43.39 ± 8.61 B	0.669 ± 0.12	2.36 ± 0.25
LSD	34.56	36.85	0.48	1.26
ANOVA *(P-value)*	0.035*	0.041*	0.968 NS	0.286 NS

In contrast, the result of Ghoraishian and colleagues (2006) showed that (53.53%) of the patients were at age group (20-39) years.

The explanation of high incidence at this age group may be attributed to the activity of thyroid gland which closely linked with ovary; the thyroid is itself dependent on direct and indirect stimulation from the ovary to discharge its own function (Rani *et al*., 2016).

The incidence rates for males and females are mostly similar in many studies as well in the present study which showed females predominance (93.33%) which is comparable with previous studies. Ghoraishian and colleagues (2006) showed that (88.04%) of hypothyroidism patients were female and (11.96%) were male. Similarly, Unnikrishnan *et al.* in (2013) that displayed significantly higher proportion of females (15.86%) versus males (5.02%).

In (2011) Santin and Furlanetto indicated that hypothyroidism is more prevalent in women especially among puberty and menopause. On the other hand, Sarfo-Kantanka and coworkers (2017) illustrated that autoimmune disorders generally, including thyroid autoimmunity, were commonly associated with female gender compared to males, due to the role of estrogen as an immune-modulator. So, the result of this study is found to be confirming the findings of many other studies which reported that the incidence of hypothyroidism in females is more than that in males and there is suggestion that female hormones such as estrogen hormone have a role in the pathogenesis of thyroid diseases; through its indirect effect on thyroid gland by increasing the thyroxin binding globulin (Santin and Furlanetto, 2011).

There were evidence that estrogen may have direct actions on human thyroid cells by estrogen receptor-dependent mechanisms, modulating proliferation and function (Sarfo-Kantanka *et al*., 2017).

Serum and salivary levels of antibodies

Table (2) demonstrated the levels of antibodies in hypothyroid patients without periodontitis and hypothyroid with periodontitis for TPO- Ab (90.55 ± 20.42 and 121.09 ± 26.96 IU/ml) and for Tg- Ab (132.84 ± 41.56 and 131.16 ±41.16 IU/ml) respectively, are significantly higher ($P<0.05$) as compared to control group of TPO- Ab (35.42 ± 8.42) and of Tg- Ab (43.39 ± 8.61); whereas there are no significant differences ($P>0.05$) between two patients groups hypothyroid without periodontitis and hypothyroid with periodontitis in TPO-Ab (90.55 ± 20.42 and 121.09 ±26.96) and Tg-Ab (132.84 ± 41.56 and 131.16 ± 41.16) respectively.

Respecting to salivary levels of TPO-Ab and Tg-Ab, there no significant differences ($P>0.05$) between groups of hypothyroid without periodontitis and hypothyroid with periodontitis in the TPO-Abs levels (0.703 ± 0.12 and 0.779 ± 0.21) and Tg-Ab levels (2.70 ± 0.24 and 3.55 ± 0.67) are increased non-significantly ($P>0.05$) than that in control groups.

The results of present study are similar with previous studies; Joshi *et al.* (2011) demonstrated that serum levels of thyroid antibodies (TAbs), TPO-Ab and Tg-Ab, in primary hypothyroid patients may be elevated as autoimmune thyroiditis or remain in normal titer as thyroid dysfunction. In addition, other studies by Ghoraishian *et al.* (2006) and Shinto *et al.* (2010) explained that the levels of TAbs in serum of hypothyroid patients with autoimmune thyroiditis are increased significantly than control, and more patients with thyroiditis have elevated serum anti-TPO than anti-Tg-Ab concentrations. Similarly, studies of Bjoro *et al.* (2000) and Ito *et al.* (2006)revealed that the large majority of patients with hypothyroidism were TPO-Ab positive; this indicates that autoimmunity is an important factor in hypothyroidism.

Whereas, Legakis *et al.* (2013) displayed that positive anti TPO-Abs were detected more than anti Tg- Ab in hypothyroid population and the positivity in both TAbs was correlated with abnormally high TSH concentrations, especially in the female population.

The development of antibodies to TPO, Tg and TSH-R is the main hallmark of AITD. Circulating T-Lymphocytes are increased in AITD and thyroid gland is infiltrated with T-Cells. However, wide varieties of cytokines are produced by infiltrated immune cells, which mediate cytotoxicity leading to thyroid cell destruction (Zimmermann, 2009).Nevertheless, there is little evidence that both Abs have a prime role in the pathogenesis of HT and it is far more likely that both T-cell mediated cytotoxicity and activation of apoptotic pathways influence the disease outcome. Yet, TAbs serve as a useful marker for the diagnosis of thyroid autoimmunity; as well, TPO-Abs in HT are present in nearly all (>90 %) patients, while Tg-Abs can be detected in approximately 80% (Zimmermann, 2009; Vanderpump, 2011).

The researchers Van-Herle and coworkers (1989) established that Tg crosses the blood/salivary gland barrier in all groups studied but is quantitatively not important. Tg-Ab also crosses the blood/salivary barrier, the concentration in saliva is however not strictly proportional to the Tg-Ab concentrations in serum. On the other hand, Shah *et al.* (1978) found that salivary thyroglobulin is parallel with serum thyroglobulin levels, in patients with thyroid cancer.

The principal biochemical characteristic of the disease is the presence of TAbs in the patients' sera against two major thyroid antigens, TPO and Tg. TPO antigen, located at the apical membrane of the thyrocyte, is essential for thyroid hormone synthesis, catalysis of iodine oxidation, iodination of tyrosine residues in Tg and coupling of the iodothyrosines into T_4 and T_3. The THs are synthesized on Tg, a large glycoprotein within thyroid follicles, which also serves as the storage for THs (Zaletel, 2007). Abs against TPO (TPO-Abs) and Tg (Tg-Abs) are of immunoglobulin G class (IgG), both showing high affinity for their respective antigens. Unlike Tg-Abs, TPO-Abs can activate complement and form terminal complement complexes within the thyroid gland which are able to cause damage to thyroid cells due to antibody-dependent cell cytotoxicity (ADCC) (Swain *et al.*, 2005; Zaletel and Gaberšček, 2011).

Conclusion

The current findings suggest that the presences of thyroid antibodies TPO-Ab and Tg-Ab which may contribute in the pathogenesis of hypothyroidism in autoimmune state. However, salivary concentration of these antibodies cannot reflect the concentration the in serum.

Conflicts of interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

References

Ajeena IM. 2013. Prevalence of Neuromuscular Abnormalities in Newly Diagnosed Patients with Thyroid Dysfunction. American Journal Research Communication **1(3),** 79-88.

Al-Shaibani AB, Sanad B, Al-A'araji SB, Al-Mofarji ST. 2014. Studying Association between

Thyroid Disorders and Helicobacter pylori infection in Iraqi Patients.Baghdad Science Journal **11(4),** 1528- 1514.

Bjoro T, Holmen J, Krueger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L, Brochmann H. 2000. Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population.The Health Study of Nord-Trondelag (HUNT).European Journal Endocrinology **143**, 639-647.

Chiappelli F, Iribarren F, Prolo P. 2006. Salivary biomarkers in psychobiological medicine. Bioinformation**8 (1),** 331-334.

DeRuiter J. 2002. Thyroid Hormone Tutorial: Thyroid Pathology. Endocrine Module- Thyroid Section, p 1-29.

Ghoraishian SM, Moghaddam SH, Afkhami-Ardekani M. 2006. Relationship between Anti-Thyroid Peroxidase Antibody and Thyroid Function Test. Iran Journal Immunology **3(3),** 146-149.

Gomes FIF, Aragão MGB, Barbosa FCB, Bezerra MM, Pinto VPT, Chaves HV. 2016. Inflammatory Cytokines Interleukin-1β and Tumour Necrosis Factor-α - Novel Biomarkers for the Detection of Periodontal Diseases: a Literature Review. Journal Oral Maxillofacial Research **7(2),** 1-10.

Holsinger FC, Bui DT. 2007. Anatomy, Function, and Evaluation of the Salivary Glands. In: Myers EN, Ferris RL (eds.). Salivary gland disorders. **XVI,** Springer.
http://www.springer.com.

Humphrey S, Williamson R. 2001. A review of saliva: normal composition, flow and function. Journal Prosthetic dentistry **85(2),** 162-168.

Ito C, Watanabe M, Okuda N, Watanabe C, Iwatani Y. 2006. Association between the Severity of Hashimoto's Disease and the Functional +874A/T Polymorphism in the Interferon-γ Gene. Endocrine Journal **53(4),** 473-478.

Javaid MA, Ahmed AS, Durand R, Tran SD. 2016. Review Article: Saliva as a diagnostic tool for oral and systemic diseases.Journal Oral Biology Craniofacial Research **6(1),** 67-76.

Joshi SR. 2011. Laboratory Evaluation of Thyroid Function. JAPI **59,** 14-20.

Legakis I, Manousaki M, Detsi S, Nikita D. 2013. Thyroid Function and Prevalence of Anti-Thyroperoxidase (TPO) and Anti- Thyroglobulin (Tg) Antibodies in Outpatients Hospital Setting in an Area with Sufficient Iodine Intake: Influences of Age and Sex. Acta Medica Iranica **(1),** 25-34.

Nagler R. 2008. Saliva Analysis for Monitoring Dialysis and Renal Function. Clinical Chemistry **54(9),** 1415-1417.

Pushparani DS. 2015. Role of Cytokines in Periodontal Wound Healing Process - A Review. Pharmaceutical Analytical Chemistry **1(1),** 1000106.

Rani AS, Rekha DB, Reddy GA. 2016. Study of Hypothyroidism in Women with Abnormal Uterine Bleeding.Journal of Dental and Medical Sciences **15(4),** 12-18.

Santin AP, Furlanetto TW. 2011. Role of Estrogen in Thyroid Function and Growth Regulation: Review Article. Journal Thyroid Research **2011,** 1-7.
http://dx.doi.org/10.4061/2011/875125

Sarfo-Kantanka O, Sarfo FS, Ansah EO, Yorke E, Akpalu J, Nkum BC, Eghan B. 2017. Frequency and determinants of thyroid autoimmunity in Ghanaian type 2 diabetes patients: a case-control study. BMC Endocrine Disorders **17(2),** 1-8.

SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary.NC

USA.

Shah D, Dandekar S, Ganatra RD. 1978. Thyroglobulin levels in serum and saliva of patients with differentiated thyroid carcinoma. Proceedings of the Indian Academy of Sciences -Section B**87 (7),** 169-175.

Shinto AS, Pachen L, Sreekanth TK, George D. 2010. Prevalence of Antithyroid Antibodies in Histologically Proven Autoimmune Thyroid Diseases and Correlation with Thyroid Dysfunction in South India. Thyroid Science **5(9),** 1-5.

Silva LM, Chavej J, Canalli MH, Zanetti CR. 2003. Determination of IgG sub classes and avidity of antithyroid peroxidase antibodies in patients with subclinical hypothyroidism- a comparison with patient with overt hypothyroidism. Hormone Research **59(3),** 118-24.

Swain M, Swain T, Mohanty BK. 2005. Autoimmune Thyroid Disorders- An Update.Indian journal clinical biochemistry **20(1),** 9-17.

Unnikrishnan AG, Kalra S, Sahay RK, Bantwal G, John M, Tewari N. 2013. Prevalence of hypothyroidism in adults: An epidemiological study in eight cities of India. Indian Journal Endocrinology and Metabolism **17(4),** 647-652.

Van Herle AJ, Rosenblit PD, Van Herle TL, Van Herle P, Greipel M, Kellett K. 1989. Immunoreactive thyroglobulin in sera and saliva of patients with various thyroid disorders: Role of autoantibodies. Journal Endocrinological Investigation **12(3),** 177-182.

Vanderpump MPJ. 2011. The epidemiology of thyroid disease. British Medical Bulletin **99,** 39-51.

Vanderpump MPJ, Tunbridge MJ. 2002. Epidemiology and Prevention of Clinical and Subclinical Hypothyroidism. Thyroid **12(10),** 839-847.

Wiersinga WM. 2016. Clinical Relevance of Environmental Factors in the Pathogenesis of Autoimmune Thyroid Disease. Endocrinol Metabolism **31,** 213-222.

Zahid TM, Bing-Yan W, Cohen RE. 2011. The Effects of Thyroid Hormone Abnormalities on Periodontal Disease Status. Journal of the International Academy of Periodontology **13(3),** 80-85.

Zaletel K. 2007. Determinants of thyroid autoantibody production in Hashimoto's thyroiditis. Expert Review Clinical Immunology **3(2),** 217-223.

Zaletel K, Gaberšček S. 2011. Hashimoto's Thyroiditis: From Genes to the Disease. Current Genomics **12(8),** 576-588.

Zimmermann MB. 2009. Iodine Deficiency-Review. Endocrine Reviews **30(4),** 376–408.