Iraqi Journal of Biotechnology, May 2018, Vol. 17, No. 1, 60-66

Correlation between thyroid hormones and anti-TSHR Ab in graves' disease

Sara M. M. Fawzi [1] , **Ismail A. Abdul-hassan**[1] , **Batool M. Mahdi**[2]

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.
[2]Head of HLA research unit, Al-Kindi college of medicine, University of Baghdad, Iraq.

Received: December 11, 2017 / **Accepted**: March 26, 2018

Abstract: Current study we aimed to study the correlation of anti TSH receptor autoantibody with the thyroid hormones in the Graves ' disease patients . This cross –sectional study includes 60 appearantly healthy control and 60 patients who were early diagnosed with thyrotoxicosis diagnosed as Graves ' disease by the physicians. Those patients were attended Hormonal Unite at Specialized Center for Endocrinology and Diabetes in Baghdad / Rusafa – Iraq for the period between May- 2016 to January - 2017. Hormonal estimation including T3, T4 and TSH and immunological study including anti-TSHR antibody. According to the results, parameters (T4, TSH, anti TSHR) showed no significant difference between male and female (P value were 42.8, 0.26 and 4.17 respectively), thus , sex had no effect on development of disease, no significant differences between (T3 ,T4,TSH) regarding to smoking (p value = 2.066 ,45.78, 0.235 respectively) , but there is significant difference in Anti TSHR between smokers and non-smokers. there is differences between levels of (T3,T4,TSH and Anti TSHR Ab) regarding to age groups , in patients age less than 40 years , there is a highly significant difference with T3 ,T4 and Anti TSHR Ab. There is correlation between Anti-TSHR with T3 and T4 showed highly Significant positive (r= +0.96, +0.75 respectively) , in contrast there is a highly significant negative between Anti-TSHR Ab and TSH (r= -0.66) . Measurement of Anti TSHR Ab in patients with thyrotoxicosis in a blood can help to establish the diagnosis and sometimes to predict the clinical course and response to treatment.

Keywords: autoimmune, Graves' disease, TSH receptor Ab.

Corresponding author: should be addressed (Email: sara.alkhayat85@gmail.com).

Introduction:

Toxic diffuse goiter affects the thyroid gland leading to hyperthyroidism and enlarged thyroid. Patients usually complain from irritability, muscle weakness and tachycardia. Many studies revealed that thyroid disorders could be due to variable genetic and hormonal factors (1,2,3,4,5,6). It is an autoimmune thyroid diseases (AITD) result from a dysregulation of the immune system that attack thyroid gland. It is a T cell-mediated organ-specific autoimmune disorder (7). The AITD comprise two main clinical presentations: Graves' disease (GD) and Hashimoto's thyroiditis (HT), both characterized by lymphocytic infiltration of the thyroid parenchyma (8). The clinical hallmarks of GD and HT are thyrotoxicosis and hyperthyroidism. Graves' disease characterized by infiltration of the thyroid by T and B cells directed against thyroid antigens, leading to production of thyroid stimulating hormone receptor stimulating antibodies (TSHR - SAbs), which induce biochemical and clinical hyperthyroidism (9). Autoantibodies activate the thyrotropin stimulating hormone receptor (TSHR) and increase target organ activity, leading to thyroid hyperplasia, increased thyroid hormone secretion, and clinical thyrotoxicosis (10). Graves' hyperthyroidism results

from the production of unique IgG antibodies that bind to and activate the thyroid-stimulating hormone (TSH) receptor on the surface of thyroid follicular cells(11). This activation stimulates follicular cell growth, causing diffuse thyroid enlargement and increased production of thyroid hormones with an increase in the fraction of triiodothyronine (T3) relative to thyroxine (T4) (12). Like other autoimmune diseases, Graves' disease is most likely caused by a combination of genetic and environmental factors that may also determine the long-term prognosis of the disease.(13). The Aim is to study the effect of anti TSH receptor autoantibody in the development of Graves ' disease.

Materials and methods:

This cross –sectional study includes 60 apparently healthy control and 60 patients who were early diagnosed with thyrotoxicosis; increased in T3 and/or T4 and decrease in TSH level and diagnosed as Graves ' disease by the physicians. Those patients were attended Hormonal Unite at Specialized Center for Endocrinology and Diabetes in Baghdad / Rusafa – Iraq for the period between May- 2016 to January - 2017. Sixty patients were GD, 48 of them were females and 12 were males, their aged were range from 25 to55 years. The other group was sixty healthy control groups who had no history or clinical evidence of hyperthyroidism. Whole blood was aspirated from GD patients and control group and the following tests were done; hormonal estimation including: T3, T4 and TSH, immunological study including anti-TSHR antibody. Total Triiodothyronine (tT3), total Thyroxine (tT4) and Thyrotropin (TSH) were done by Enzyme Linked Immune Sorbent Assay test (ELISA) according to manufacturing instructions (Monobind Inc./USA). Other test was Anti-TSH Receptor Antibodies was done by ELISA test using IMTEC- TSH Receptor - Antibodies ELISA Kit, Human/ Germany.

Statistical analysis:

It was done using System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and least significant difference –LSD test was used to significant compare between means in this study. P value less than 0.05 was considered significant. (14)

Results:

In current study, table (1) showed the differences between control and patients regarding hormones showed a highly significant differences (P-value=0.0001) for T3 control (1.597± 0.04) and patient (5.480 ± 0.47), T4 (93.67 ± 11.87) (182.78 ± 13.54), TSH (2.693 ± 0.150) (0.681 ± 0.14), AND Anti TSHR Ab (0.386 ± 0.02), (12.11 ± 1.15). These results showed high level of T3 and T4 with low level of TSH.

Table (1): comparison between Graves' disease and control group.

Hormones	Mean ± SE		P-value
	Control (No. = 60)	Patients (No. = 60)	
T3	1.597 ± 0.04	5.480 ± 0.47	0.0001 **
T4	93.67 ± 11.87	182.78 ± 13.54	0.0001 **
TSH	2.693 ± 0.15	0.681 ± 0.14	0.0001 **
Anti TSHR	0.386 ± 0.02	12.11 ± 1.15	0.0001 **
** (P<0.01)			

This study showed there is no significant difference (P=1.983) between male and female regarding T3 hormone (4.26 ± 1.13) in male and (5.78 ± 0.51) in females. Other parameters (T4, TSH, anti TSHR) showed no significant difference between male and female (P value were 42.8, 0.26 and 4.17 respectively), thus, sex had no effect on development of disease as shown in table (2).

Table (2): Effect gender on levels of thyroid hormones.

Sex	Mean ± SE			
	T3	T4	TSH	Anti-TSHR
Male (No. = 12)	4.26 ± 1.13	155.82 ± 34.88	0.729 ± 0.23	9.50 ± 2.51
Female (No. = 48)	5.78 ± 0.51	189.52 ± 14.55	0.669 ± 0.17	12.76 ± 1.29
LSD value	1.983 NS	42.894 NS	0.260 NS	4.176 NS
NS: Non-significant.				

This study showed that there is no significant differences between (T3, T4,TSH) regarding to smoking (p value = 2.066, 45.78, 0.235 respectively) , but there is significant difference in Anti TSHR between smokers and non-smokers. (p-value = 2.056).table (3).

Table (3): Effect of smoking on levels of thyroid hormones.

Smoking	Mean ± SE			
	T3	T4	TSH	Anti-TSHR
Smoker	4.45 ± 0.89	155.12 ± 26.45	0.583 ± 0.18	9.82 ± 1.91
No smoker	5.85 ± 0.55	192.84 ± 15.67	0.716 ± 0.18	12.94 ± 1.40
LSD value	2.066 NS	45.78 NS	0.235 NS	2.056 *
* (P<0.05), NS: Non-significant.				

Table (4) demonstrated that there is differences between levels of (T3, T4, TSH and Anti TSHR Ab) regarding to age groups , in patients age less than 40 years , there is a highly significant difference with T3 ,T4 and Anti TSHR Ab .

Table (4): Effect of age group on levels of thyroid hormones.

Age group (year)	Mean ± SE			
	T3	T4	TSH	Anti-TSHR
Less than 40	8.03 ± 0.58 a	250.66 ± 19.56a	0.287 ± 0.16 c	18.29 ± 1.54 a
40-50	3.88 ± 0.63 b	135.16 ± 13.09 b	0.707 ± 0.20 b	8.10 ± 1.38 b
More 50	3.54 ± 0.95 b	142.91 ± 36.99 b	1.48 ± 0.45 a	7.73 ± 2.53 b
LSD value	2.337 **	63.189 **	0.273 *	4.309 **
* (P<0.05), ** (P<0.01).				

There is correlation between Anti-TSHR with T3 and T4 showed highly Significant positive (r= +0.96, +0.75 respectively), in contrast there is a highly significant negative between Anti-TSHR Ab and TSH (r= -0.66) as shown in table (5).

Table (5): Correlation between T3,T4,TSH and Anti TSHR Ab in Graves' disease patients.

Parameters	Correlation coefficient- r	Level of sig.
Anti-TSHR & T3	+0.96	**
Anti-TSHR & T4	+0.75	**
Anti-TSHR & TSH	-0.66	**
**** (P<0.01).**		

Discussion:

Graves' disease results from the production of IgG antibodies that bind to and activate the thyroid-stimulating hormone (TSH) receptor on the surface of thyroid follicular cells leading to diffuse thyroid enlargement and increased roduction of thyroid hormones (14). In this study there is no significant difference (P=1.983) between male and female regarding T3 hormone and other parameters (T4, TSH, anti TSHR) in male and in females because it is the same disease pathogenesis. while this disease is more common in female than males which is in agreement with other studies demonstrated that female are more predispose to development of graves' disease due to sex hormone (estrogen) which increase the liability to autoimmune disease (16,17). Regarding, it had an effect on the development of graves' disease in this study, This is in agreement with the meta analysis done (18). concluded that smoking was not associated with toxic nodular goiter (12). In contrast, smoking was recognized as a clear risk factor for Graves' disease. For Graves' hyperthyroidism. Saad (2015)(19) showed that the percentages of smokers were (17.5 and 25) % in Graves' Disease patients and control subjects, respectively. Thus smoker had no effect on disease development. This difference with other studies may be due to sample, criteria, samples size, method used, family history. Age also had an effect on disease. (20) demonstrated that GD is most common in younger Polish age group especially when the patient had HLADRB1*03 allele. GD was diagnosed at younger age in patients with family history of this disease (21).

The study showed high level of T3 and T4 with low level of TSH. This is due to excess production of T3 and T4 accompanied by low level of TSH was believed that the source of these antibodies is immune competent plasma cells. The antibodies bind with TSHR to initiate and increase T3 and T4 synthesis and production regardless of decrease level of TSH by negative feedback mechanism which exerted by T3 and T4 on pituitary and hypothalamic axis. TSH concentrations found to be decreased, whereas, T3 and T4 were found to be increased among patients with Graves' disease. These results are in agreement with other studies (22, 23, 24), who found an increased thyroid hormone levels with suppressed TSH levels in patients with Graves' disease.

Thyroid autoantibodies are the markers of autoimmunity in autoimmune thyroid diseases (25, 26) Graves' hyperthyroidism disease which is an autoimmune disease where the antibodies to the thyroid stimulating hormone receptor (TSHR) make the thyroid gland produce too much hormone(27). TSHR autoantibodies play a direct role in the pathogenesis of AITD. Therefore (TRAb) assay is helpful in diagnosis of Graves' disease when clinical features are not

conclusive, and make as a golden diagnostic marker for Graves' disease.
The result showed that the Anti-TSHR Ab is high level in patients than apparently healthy people (p- value = 0.0001) (0.386 ± 0.02 *versus* 12.11±1.15) , This result agreed with other studied which conducted by Alfadil *et al*.,(28),who explained that the significant increase in mean of Anti TSHR Ab in patients with graves when compared with both patients with hyperthyroidism and control group .Amballi, reported that elevation of Anti TSHR Ab in untreated Grave's disease 95% of patients were TRAb positive while 15% of patients diagnosed as nodular toxic goiter were Anti TSHR Ab positive(29).A study by Pedersen,(30) reported that 90% of patients were TRAb positive due to hyperthyroidism of Graves' disease that was distinguished clinically from the presence of a painless diffuse goiter(31).

There is a positive correlation between Anti-TSHR with T3 and T4 while negative correlation with TSH (r= -0.66) as shown in table-5-. This was agreed with KHen,(2007) (32),who showed that the correlation between Anti and degree of thyrotoxicosis (T4 at diagnosis) P<0.01. Chen *et al*., (33) was presented TSHR autoantibodies detected in more than 90% of untreated Graves patients, and TSHR Ab titer correlate closely with the severity of hyperthyroidism. The relationship between the TSH receptor antibodies and the excess production of thyroid hormones suggests the activation of B cells and dominance of humoral immune responses in patients with Graves' disease (34). In Graves' disease, autoantibodies bind to the receptor and mimic TSH action and stimulate thyroid cells. This leads to hyperthyroidism and abnormal overproduction of thyroid hormone (35).Mechanism of TSHR-autoantibodies production is more or less clear but a susceptibility gene, which is linked to their production, is still unknown. Genetic studies showed no linkage between the TSHR gene and Graves' disease (36). The primary cytokines secreted from Th1 cells include IL-2, IFNγ, and TNF-β, overproduction of these cytokines from activated Th1 cells can induce excessive B-cell activation and autoantibody production (34,35).

Conclusion:

Measurement of Anti TSHR Ab in patients with thyrotoxicosis in a blood can help to establish the diagnosis and sometimes to predict the clinical course and response to treatment. Although the TRAb assay has become more reasonable, cost remains an important factor when considering it as routine use. It is a niche investigation with the development of automated and less labor intensive assay that facilitate more widespread use of TRAb test in patients with hyperthyroidism.

Recommendation:

- Everyone with Graves ' disease should get treatment immediately after disease diagnosis.
- **A**dults should be test for (TSH, T3, T4) .

References:

1- AL-Faisal A.H.M. , AL-Ramahi I.J. , Abudl-Hassan I.A. 2012. Micronucleous frequency among Iraqi thyroid disorder patients. Comparative Pathology Journal , 21(5) :1671-1677.

2- AL-Ramahi I. J., AL-Faisal A. H. M., Abudl-Reda I., Aouda N.3, AL-Atar R.

and Barusrux S. 2012. Screening of g.IVS5+1G to a mutation of *TG* gene and thyroid hormone level among Iraqi thyroid disorders. Journal of Medical Genetics and Genomics 4(1):1–5.

3- AL-Faisal A.H.M. , AL-Ramahi I.J. , Abudl-Hassan I.A. ,Hamdan A.T. and Barusrux S. 2012. Detection of heterozygous c.1708C>T and c.1978C>G thyroid peroxidase (TPO) mutations in Iraqi patients with toxic and non toxic goiter. Comparative Pathology Journal , 21(3) : 1572-1581.

4- AL-Faisal A.H.M. , AL-Ramahi I.J. , Abudl-Hassan I.A. 2012. Detection of homozygous G.IVS34-1G>C AND C.886C>T mutations among toxic goiter and thyroid cancer patients in Iraq. The Iraqi Journal of Biotechnology, 11(2):414-421.

5- Al-Faisal, A.H.M. , Abdul-Hassan, I.A. , Al-Ramahi, I. J. , and Barusrux, S. 2013. Screening of TPO gene c.1708C<T and c.1978C<G mutations in Iraqi patients with hypothyroidism and thyroid cancer. First International Scientific Conference of Zakho University, 23-25 April.

6- Abdul Hussan,I.A., AlRamahi,I.J. and AlFaisal,A.H.M. 2013. Detection of TG and TPO genes compound mutations associated with thyroid carcinoma, toxic goiter and hypothyroidism in Iraqi patients. J.Med.Sci., On line 2013,1-8.

7- Swain, M.; Swain, T. and Mohanty, B.K. (2005). Autoimmune Thyroid Disorders. indian journal of clinical biotechnology 20(1): 9-17 .

8- Antonelli, A.; Ferrari, S.M.; Corrado A.; Di Domenicantonio, A. and Fallahi P. (2014). Journal autoimmunity reviews., 14(2):174-180.

9- Melmed, S.; Polonsky, K.S.; Larseen, P.R. and Kronenberg, H.M. (2016). William text book of endocrinology, 13 th edition, Chapter 12: 375 .

10- Chazenbalk, G.D.; Pichurin, P.; Chen, C.R.; Latrofa, F.; Johnstone, A.P.; McLachlan, S.M. and Rapoport, B. (2002). Thyroid-stimulating autoantibodies in Graves disease preferentially recognize the free A subunit, not the thyrotropin holoreceptor. J. Clin. Invest.; 110(2): 209-217.

11- Menconi, F.; Marcocci, C. and Marinò, M. (2014). "Diagnosis and classification of Graves' disease". Autoimmunity Reviews. 13 (4–5): 398–402.

12- SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

13- Girgis, D.C.M.; Champion, B.L. and Wall, J.R. (2011). Current Concepts in Graves' Disease.Ther Adv Endocrinol Metabolism., 2(3): 135–144.

14- Lichiardopol1, C. and Mota, M. (2009). The Thyroid and Autoimmunity. Rom. J. Intern. Med.; 47 (3): 207-215.

15- Hawazen, A. (2008). Thyroid Disorders In Makkah, Saudi Arabia. *Ozean Journal of Applied Sciences*, 1(1): 55-58.

16- Vestergaard, P.; Rejnmark L.; Weeke, J.; Hoeck, H.C.; Nielsen, H.K.; Rungby J.; Laurberg, P. and Mosekilde, L. (2002). Smoking as a risk factor for Graves' disease, toxic nodular goiter, and autoimmune hypothy- roidism. Thyroid. 12(1):69-75.

17- Saad, S.A. (2015). The association between the polymorphisms in the CTLA-4 gene and the risk of Graves' disease., Iraq,Baghdad university, the Council of Genetic Engineering and Biotechnology Institute for Postgraduate Studies. (56-57).

18- Jurecka-Lubieniecka B.; Ploski, R.; Kula, D.; Krol, A.; Bednarczuk, T.; Kolosza, Z.; Tukiendorf, A.; Szpak-Ulczok S.; Stanjek-Cichoracka, A.; Polanska, J. and Jarzab, B. (2013). Association between Age at Diagnosis of Graves' Disease and Variants in Genes Involved in Immune Response. PLoS One. 8(3):e59349.

19- Manji, N.; Carr-Smith, J.D.; Boelaert, K. Allahabadia, A.; Armitage, M.; *et al*. (2008). Influences of age, gender, smoking, and family history on autoimmune thyroid disease phenotype. J. Clin. Endocrinol & Metabolism. 91: 4873–880.

20- Magdy, H.; El-Maghraby, T.; Khalafallah, K.; Omar, W.; Grace, B.D. and Al-Nahhas, A. (2007). Clinical significance of metabolic superscan in patients with hyperthyroidism. Nuclear Medicine, National Cancer Institute, Cairo University, Egypt. Nuclear Medicine Review: 10(2): 76-81.

21- Fountoulakis, S.; Vartholomatos, G.; Kolaitis, N.; Frillingos, S.; Philippou, G. and Tsatsoulis, A. (2008). Clinical Study of Differential expression of Fas system apoptotic molecules in peripheral

lymphocytes from patients with Graves' disease and Hashimoto's thyroiditis. *European Journal of Endocrinology*; 158: 853–859.

22- Veeramuthumari, P.; Isabel, W. and Kannan, K. (2011). A Study on the Level of T3, T4, TSH and the Association of A/G Polymorphism with CTLA-4 Gene in Graves' Hyperthyroidism among South Indian Population. *J. Clin. Biochem*, 26(1): 66–69.

23- AL-Naqdy, A.; Kutty, I.; AL-Harthi, S.; AL-Buloshi, M. and AL-Maskari, M. (2003). Anti-thyroid microsomal antibodies in thyroid disorders. Bahrain Medical Bulletin; 25(3).

24- Elmugadam, A.A.; Elobied, E.A.; Mustafa H.A.; Elfadil, G.A. and Makeen A.M. (2014). Anti-TPO and Anti-Tg Antibodies in Sudanese Patients with Thyroid Diseases and Association of HLA Class- II with Graves' disease.Journal of American Science;10 (2): 39-42.

25- Kadhum, E.W. and Hassan, A.J. (2014). Study of some aspects of autoimmune response for patients of thyroiditis. J. pharmacy and Biological sciences; 9(6): 01-04.

26- Amballi, A.A., (2007). Thyrotocosis. Middle-East journal of scientific; 2(3-4): 98-103.

27- Pedersen, L.I.B. (2007). Measurements of TSH receptor antibodies in differential diagnosis. Ligand Assy; 12 (1): 62-65.

28- Elfadil, G.A.; Eltahir, I.E.M. and Elmugadam, A.A. (2014). Anti-TRA-Ab, Anti-TPO-Ab, and FT3 as a Biochemical Panel for Differential Diagnosis of Graves' Disease from Hyperthyroidism. Indian J. of Applied Research; 4(5): 408-410.

29- AL-Gazally, M.E.; Al-shalah, M.A. and Muttaleb, S.A. (2013). The Role of Thyrotropin Hormone Receptor Antibody (TRAb) in Distinguishing between Autoimmune and non-Autoimmune Disease. Medical Journal of Babylon; 10(1): 116-153.

30- Itoh, M.; Uchimura, K.; Yamamoto, K.; Makino, M.; Imamura, S.; Kobayashi, T.; *et al.* (2002). Distinctive response of thyroid- infiltrating mononuclear cells to B cell activation through CD40 and interleukin-4 in Graves' patients. cytokine;19(3): 107–114.

31- Chistiakov, D.A. (2003). Thyroid-stimulating hormone receptor and its role in Graves' disease. Mol. Genet Metab.; 80(4):377-88.

32- Khin, S.M.; Gayathridevisa, S.N.; Ian, M.F.; Mark, G.; Diana, W.; Khris, C. and Helen, S. (2007). Use of Anti-Thyroid Hormone Receptor antibody (TRAB) in Graves' Disease. Endocrine. Abstracts. 13: 81.

33- Chen, C.R.; Pichurin, P.; Nagayama, Y.; Latrofa, F.; Rapoport, B. and McLachlan, S.M. (2003). The thyrotropin receptor autoantigen in Graves' disease is the culprit as well as the victim. J. Clin. Invest.; 111(12): 1897–1904.

34- Brand, O.J.; Barrett, J.C., Simmonds M.J.; Newby, P.R.; McCabe, C.J.; Bruce C.K.; *et al.* (2009). Association of the thyroid stimulating hormone receptor gene (TSHR) with Graves' disease. Hum Mol. Genet.; 18(9):1704-1713.

35- Hampe, C.S. (2012). B Cells in Autoimmune Diseases. Review Article; Scientifica; 18.