

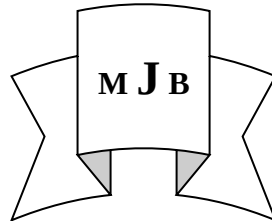
The Role of Thyrotropin Hormone Receptor Antibody (TRAb) in Distinguishing between Autoimmune and non-Autoimmune Disease

Moaed Emran Al-Gazally

Muhammed Abbas Al-shalah

Saif Ali Muttaleb

College of Medicine, University of Babylon, Hilla, Iraq.



Abstract

The present study was intended to investigate Thyrotropin Receptor Antibody (TRAb) level in hyperthyroidism. A total of 65 patients and 25 apparently healthy as a controls were assessed. Twenty-seven of the 65 patients had Graves' disease, twenty-one had toxic nodular goitre and the remaining group had toxic nodule. In comparison with the control group, patients with diffuse goitre showed a highly significant increase in TRAb ($P < 0.01$) significant increase in TRAb ($p < 0.05$) in nodular toxic goitre, while non-significant ($p > 0.05$) found in nodule toxic. Thyroid function tests appear significant increase ($P < 0.01$) T4, T3 while significant decrease in thyroid stimulating hormone TSH concentrations. The incidence of positive TRAb assay in diffuse goitre group was 89%, whereas 24% were positive in toxic nodular goitre group and 6% in toxic nodule. It can be concluded that as a practical method, TRAb assay may be useful in making a differential diagnosis of Graves' disease rather than other autoimmune thyroid diseases (toxic nodular and toxic nodule).

Keywords: Thyrotropin hormone receptor antibody (TRAb), Toxic multinodular TMN, Toxic nodule (TN), T4, T3.

الخلاصة

تضمنت الدراسة معرفة مستوى الأضداد الذاتية للمستقبلات للهرمون المحفز للغدة الدرقية. حيث شملت الدراسة 65 مريضاً مصاباً بفقر إفراز هرمونات الغدة الدرقية و 25 شخصاً ظاهرياً أصحاء كمجموعة سيطرة. 27 من المرضى لديهم المرض المناعي التلقائي، و 21 من المرضى لديهم غدة درقية متعددة العقد السامة والمجموعة الباقية لديها عقدة سامة. بالمقارنة مع مجموعة السيطرة، أظهرت نتائج المرضى المصابين بالمرض المناعي التلقائي المنتشر (Graves' disease) وجود زيادة عالية المعنوية في تركيز المضاد الذاتي للمستقبل هرمون الغدة الدرقية ($P < 0.01$) كذلك زيادة معنوية في التركيز للمرضى المصابين بمتعدد العقد السامة ($P < 0.05$)، بينما لا يوجد فرق معنوي للمرضى المصابين بالعقدة السامة. اختبار هرمونات الغدة الدرقية يظهر زيادة عالية المعنوية في تركيز كل من هرمون T3 و T4 ($P < 0.01$) بينما TSH اظهر نقصان عالية المعنوية بالتركيز ($P < 0.01$).

وجد أن نسبة 89 % من الأضداد الذاتية للمستقبلات هرمونات الغدة الدرقية موجودة في مجموعة المرض المناعي التلقائي ونسبة 24 % لمجموعة المرضى متعدد العقد السامة ونسبة 6 % لمجموعة مرضى العقدة السامة. نستنتج من ذلك يمكن استخدامه كطريقة عملية في التشخيص بين المرض المناعي التلقائي (Graves' disease) عن الأمراض الغير المناعية الأخرى المتمثلة بالعقيدات السامة والسامة متعددة العقيدات.

Introduction

Thyrotoxicosis represents the clinical syndrome that results from exposure to elevated levels of circulating thyroid hormones. Hyperthyroidism is used to describe thyrotoxicosis resulting from

overproduction of TH by thyrocytes, with GD the commonest cause [1].

This disorder had also been found to affect women five times more than men [2]. There are several causes of hyperthyroidism one of which an autoimmune disorder, its etiology involves the production an antibodies

against the thyroid stimulating hormone (TSH) receptors, TSHR that result in excess production of T3 and T4 [3].

Moreover, high level of TH can also result from autonomous production by solitary or multiple thyroid nodules, which secrete thyroid hormones autonomously. In addition, excessive TSH secretion by pituitary tumors resulted in higher stimulation to thyroid gland [4].

The overproduction of human chorionic-gonadotropin hormone (HCG) during pregnancy competes with TSH in binding with TSHR [5].

Thyroiditis, an inflammatory state of thyroid gland also resulted in liberation of stored hormones (T3 and T4) into blood circulation [6]. Finally, the other miscellaneous causes represent a little incidence in hyperthyroidism such as metastatic thyroid carcinoma, and struma ovarii [7]. Thyrotoxicosis is retained because hyperthyroidism i.e. symptoms due to a raised level of circulating thyroid hormone, is not responsible for all manifestations of the disease. Clinical types are [8]:

Diffuse toxic goiter (CD), toxic nodular goiter, toxic nodule and Hyperthyroidism due to rarer causes.

The signs of hyperthyroidism include: muscle weakness especially upper arms and thighs, shaking hands, speeding up of heartbeat from a normal rate of 70 or 80 to well over 100 beats per minute, and diarrhea exists [9].

GD is unique among autoimmune conditions in that the immune response, rather than being progressively destroyed by it stimulate the target tissue. This, coupled with the early identification of circulating thyroid-stimulating autoantibodies as the immunological hallmark of the condition, has led to Graves' disease acting as a paradigm for research into autoimmune endocrinopathies [10].

GD patients have been shown to different clones of TSHR autoantibodies directed at different locations of the TSHR [11]. The actions of the different

TRAbs are thought to vary due to the different binding sites. Consequently there are different types of TRAbs: stimulating, blocking and neutral [12].

In the case of GD, the patient's immune system is triggered to produce antibodies towards their own thyroid. TSAbs are one of the functional autoantibodies directed against TSHR [13].

TSH receptor antibodies are useful in the following clinical conditions. Diagnosis of euthyroid Graves. Prediction of relapse during antithyroid drug therapy in Graves. Prediction of transient neonatal hypothyroidism in mothers with blocking receptor antibodies or neonatal hyperthyroidism due to stimulating receptor antibodies [14].

A simple nodular goitre is present for a long time before the hyperthyroidism [8]. The most common cause of hyperthyroidism in those older than 40 years [15], and is very infrequently associated with eye signs. The syndrome is that of secondary thyrotoxicosis.

Patients and Methods

Patients

This study was performed at the laboratory of Biochemistry Department, College of Medicine, University of Babylon. The collection of samples was conducted during the period from 1 December 2011 until 30 June 2012 in Hilla Teaching Hospital.

The patients group who subjected in this study was (65) persons ranging from 15-61 years old, the mean $\pm$ standard deviation (SD) was (38.63 ± 10.03 years). This group comprises 13 males (20.00%), their ages ranging from 15-52 years old, the mean $\pm$ SD was (36.46 ± 10.40 years), and 52 females (80.00%) with ages ranging from 20-61 years old, and mean $\pm$ SD was (39.17 ± 9.96 years). All of those patients were subjected to the surgeon in hospital and recorded clinical symptoms and signs of

these patients were diagnosed in the laboratory.

Twenty-five apparently healthy individuals were taken as a control group. This group comprises of 6 males (24.00%) their age ranging from 19-62 years old, mean $\pm$ SD was (43.33 $\pm$ 15.76 years), and 19 females (76.00%) their age ranging from 21-57 years old, mean $\pm$ SD was (37.31 $\pm$ 8.36 years).

The present study, patients with hyperthyroidism was divided into three groups according to *UIS* techniques:

1. Diffuse goitre group: these patients showed diffuse with no echogenicity in 27 patients.
2. Toxic nodule group: These patients showed single small low echogenicity in 17 patients.
3. Toxic nodular goitre group: These patients showed multiple small low echogenicity in 21 patients.

UIS technique is useful in distinguish between diffuse group from two other groups. The study of Pederson and Aardal [16] explains that there is 70% of patient with GD exhibit low echogenicity in *UIS*. In our study 27 patient show diffuse and 38 patient show nodular goitre.

Method

TRAb was measured by using Enzyme Immunoassay method (ELISA). All statistical analyses were performed by using SPSS 15 software for Windows.

Results and Discussion

Age

The mean $\pm$ SD of age of patients with hyperthyroidism was 38.63 $\pm$ 10.03 years old with the range of 15-61 years. Age distribution of patients with hyperthyroidism is shown in fig. 1

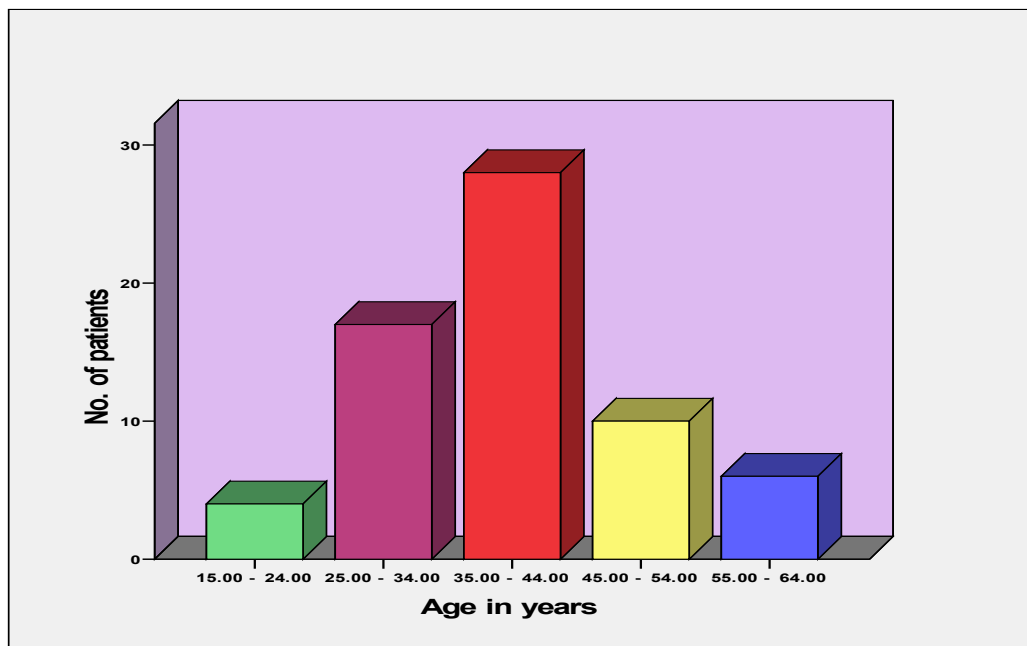


Figure 1 Age distribution of patient with hyperthyroidism.

From this figure we can see that 55 patients (83.33%) lie in the age between 25 and 54 years old. Also four patients lie in the range of 15-24 years, and six lie in the range of 55-64 years, and this is consistent with the fact that thyroid disorder may occurs at any age but it appears that the most susceptible age group to this disorder was that

ranging from 35-44 years and this result disagreement with other studies by Masinkiewicz , Burrow [17] and Bonar *et al* [18] that considered the most susceptible age group to this disorder was that ranging from 20-30 years.

Gender and hyperthyroidism

Amongst 65 patients with hyperthyroidism contribute in this study, there were 13 males and 52 females, and this represents 20 % and 80 % of patients respectively.

From the above results, it seems that females were more susceptible to such disease than males and that agrees with several studies Ali *et al* [19] Ahmed *et al* [20]. The reasons that may assist or exacerbate the female to acquire this disease could be due to sex hormones imbalance such as estrogen hormone, which is normally elevated in females during puberty and pregnancy.

Types of hyperthyroidism:

The distribution of among hyperthyroidism types for patients showed that diffuse toxic patients represent 41.53 % of patients group, while toxic nodule patients represent 26.15 % and toxic nodular goitre patients represent 32.30 %.

Hormonal measurements

The concentration of (TSH μ U/ml) in patient reduces significantly, while the concentration of (T3 nmol/l, T4 nmol/l) increases significantly as compared with normal control group ($p < 0.01$) as shown in table (1).

Table 1 Mean and SD of concentrations of TSH, T3 and T4 in patients with Control groups.

Parameters	Groups	Mean $\pm$ SD	P-value
TSH μ U/ml	Patients	0.12 $\pm$ 0.23	$P < 0.01^{**}$
	Control	0.56 $\pm$ 0.35	
T3 nmol/L	Patients	2.38 $\pm$ 0.99	$P < 0.01^{**}$
	Control	1.23 $\pm$ 0.42	
T4 nmol/L	Patients	131.93 $\pm$ 36.28	$P < 0.01^{**}$
	Control	87.45 $\pm$ 13.45	

****:** the difference is highly significant at $p=0.01$.

The data obtained from this work involved significant increase ($p < 0.01$) levels of T3 and T4 associated with significant decrease ($P < 0.01$) of TSH levels of both males and females in comparison with control subjects. From such interesting results, attention should be directed for the fact that excess production of T3 and T4 with low level of TSH. There are three explanations and reasons as we believe for such phenomenon. 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 feed back mechanism which exerted by T3 and T4 on pituitary and hypothalamic axis by Hollowell *et al* study⁽¹⁴³⁾.

The mean $\pm$ SD of TRAb U/L in patient and control are (9.81 $\pm$ 12.18) and (1.30 $\pm$ 0.80), respectively. The results show TRAb in patient increase significantly compared with control, ($P < 0.01$) in figure (2).

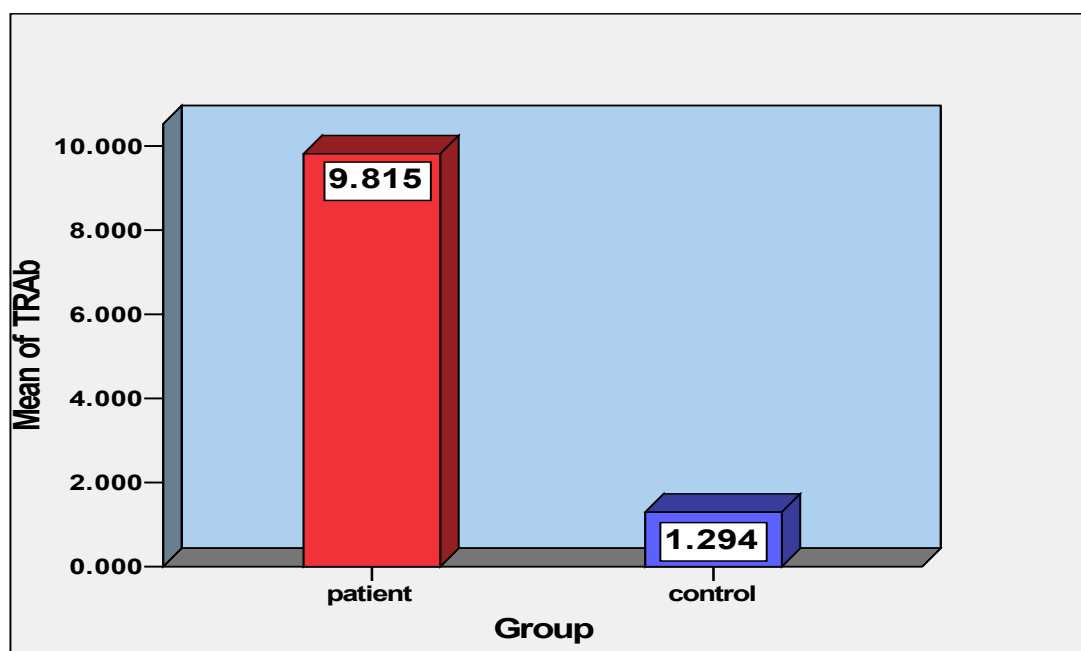


Figure 2 Mean of TRAb concentration in sera of patient and control groups.

The present study involves the effects of TRAb in these groups, which are presented in table (2).

Table 2 The effects of TRAb upon three groups for hyperthyroidism.

Parameters	Group III	Group II	Group I	Control
Control	**	*	NS	-----
Group I	**	NS	-----	NS
Group II	**	-----	*	*
Group III	-----	**	**	**

Group I = toxic nodule, Group II= nodular toxic, Group III= diffuse goitre

** : the difference is highly significant at $p=0.01$.

* : the difference is significant at $p=0.05$.

NS: Non significant at $p=0.05$.

It appears that the most susceptible diffuse group to this disorder was that 24(89%) positive from 27 patients, while toxic nodule group was 1(6%) from 17 patients, and nodular toxic group 5(24%) from 21 patients.

This result showed a highly significant increase ($P<0.001$) in diffuse patients in comparison with control subjects, while showed non-significant

($p>0.05$) in nodule patients in comparison with control subjects and found significant increase ($p<0.05$) in nodular toxic patients in comparison with control subjects.

These results agrees with other studies by A.A.A mballi [21] who reported that elevation of TRAb in untreated Grave's disease 95% of patients are TRAb positive while 15%

of patients diagnosed to have nodular toxic goitre are TRAb positive.

The study of Laurberg, Pedersen [22] who reported that 90% of patients are TRAb positive causes hyperthyroidism of Graves' disease that is distinguished clinically from the presence of a painless diffuse goiter.

TRAb is a well known marker of thyroid gland autoimmunity and may

have some predictive value in the recurrence of Graves' disease after treatment with antithyroid drugs Arqueros study [23]

Results of Mean TRAb titers and incidence of positive TRAb assay in group A, B, C and D are summarized in table (3).

Table 3 Mean TRAb titers and incidence of positive TRAb assay in group A, B, C and D.

Groups	Total	Positive n.	Percentage of Positive TRAb Titer
Group A	27	24	89 %
Group B	17	2	6%
Group C	21	5	24%
Group D	25	0.0	0.0

Group A,B,C and D = diffuse toxic goiter, nodule toxic, nodular toxic goiter and control respectively **: the difference is highly significant at $p=0.01$.

***: the difference is significant at $p=0.05$.NS: Non significant at $p=0.05$.**

It appears that the most susceptible diffuse group to this disorder was that 24(89%) positive from 27 patients, while toxic nodule group was 1(6%) from 17 patients, and nodular toxic group 5(24%) from 21 patients.

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The study of Laurberg, Pedersen [25] who reported that 90% of patients are TRAb positive causes hyperthyroidism of Graves' disease that is distinguished clinically from the presence of a painless diffuse goiter.

TRAb is a well-known marker of thyroid gland autoimmunity and may have some predictive value in the recurrence of Graves' disease after treatment with antithyroid drugs Arqueros study [26].

Although not recommended as a first line test in the diagnosis of Graves' disease, the TRAb assay is more sensitive than a homogeneous thyroid scan and a palpation of a diffusely enlarged gland (89% versus 78.1% and 74.8 %) [27] , clinical findings, the measurement of the anti TPO, and a radioiodine uptake of the thyroid cannot always give sufficient evidence to diagnose Graves' disease in some

patients, so it can be important in the diagnosis of Graves' disease [28]. So, this finding may be helpful in distinguishing these diffuse toxic from another two groups especially when clinical and laboratory findings are insufficient in confirming the diagnosis of Graves' disease.

The used TC distribution scintiscan showed difficulties in differentiation between Graves' disease and toxic nodular goitre. Previous study detected thyroid stimulating antibody in 11 of 18 (56 %) patient with TMG who showed diffuse but uneven TC distribution scintiscan, so uneven distribution of TC scintiscan not exclude autoimmune thyroid (GD) Kraiem and Glaser studies [29]. Also RAI therapy is commonly used in treatment of patient with hyperthyroidism, for patient with TMG the development of autoimmune hyper or hypothyroidism after RAI has been reported in 5% or 7% [30]. This consolidate that low echogenicity in **UIS** and uneven Tc distribution scintiscan cannot differentiations between autoimmune from non-autoimmune

The proximate cause of hyperthyroidism in patients with Graves' disease is activation of TSHR on thyroid follicular cells by (TRAb) [31]. These receptors are composed of an extracellular amino-terminal domain and a transmembrane and intracellular domain. After insertion into the cell membrane the extracellular domain can be cleaved in two places, forming an A subunit and a B subunit, which are joined by disulfide bonds. The binding site (and epitope) for TRAb and the binding site for TSH overlap each other at the amino-terminal end of the A subunit. Previous studies of the reactivity of TSAb have suggested that the antibodies bind primarily to discontinuous segments of the amino-terminal domain of the TSH receptor [32], indicating that both conformation of the receptor and its amino-acid sequence are determinants of antibody

reactivity; in other words the epitopes are conformational and structural.

It can be concluded that as a practical method, TRAb assay may be useful in making a differential diagnosis of Graves' disease from other non-Autoimmune Thyroid Diseases (AITD's), for patients on our studies.

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