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TSHR-AB ELISA KIT: A NOVEL DIAGNOSTIC KIT FOR HASHIMOTO'S AND GRAVES' DISEASE PATIENTS

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Abstract: **Background: one of the global public health problem is Thyroid gland disease. More studies are still required to investigate novel markers to specify the prognosis and recovery of the thyroid gland after both internal and external radiation exposure.**
Methodology: ELISA was used to quantitatively identify the serum level of TSHR-Ab in human blood specimens of Hashimoto's and Graves' disease and normal healthy controls.
Results: Increased levels of TSHR-Ab (P<0.01) were observed in Graves' disease patients in comparison to the normal healthy controls, it raising significantly (P < 0.01) after one year of curing with anti-thyroid drugs. In contrast, low levels of TSHR-Ab were observed in Hashimoto's disease cases, and it decreased significantly (P < 0.01) after treatment.
Conclusion: serum TSHR-Ab levels in hyperthyroidism and Hypothyroidism patients might be an effective diagnostic marker that increases the immunological response of T cell and immune system, and optimize the chance for detection and remission of autoimmune thyroid disorder

***Keywords*— thyroid-stimulating hormone antibody, thyroid-stimulating hormone, Graves disease, Hashimotos disease.**

I. INTRODUCTION

One of the common representatives in autoimmune disease is spectrum Autoimmune thyroid disease (AITD) (Ross et al., 2016). AITD is recognized usually with the existence of antithyroid peroxidase and anti-thyroglobulin autoantibodies in synchronization with thyroid hormone disparity (Fröhlich and Wahl, 2017). Respectively the most famous causes of hypothyroidism and hyperthyroidism in AITD are Hashimoto's disease and Grave's disease, (Ross et al., 2016). The common reason for autoimmune hypothyroidism is Hashimoto's thyroiditis, an autoimmune injury to the thyroid glands. Autoimmune hypothyroidism can happen because of thyroid-stimulating hormone receptor blocking antibodies (TSHR-ab), and can be hard to differentiate from Hashimoto's and Graves disease. Grave's disease is the most famous case of hyperthyroidism and is almost caused by stimulation of TSHR-ab working as an agonist for the thyroid stimulating hormone receptor (Michelle N., and Jeffrey A., 2021).

During the past fifteen years, sensitivity enhancement and specificity of thyroid test methodologies have highly influenced the clinical strategies for discovering and curing thyroid disorders. Since 1970, modern technologies in radioimmunoassay methodologies have enhanced both the specificity and sensitivity of the approach. (Joshi, 2011).

The main cause of Hyperthyroidism in Graves' disease is thyroid-stimulating autoantibodies to the TSH receptor, whereas hypothyroidism in Hashimoto's thyroiditis is comes with thyroid peroxidase and thyroglobulin autoantibodies. (McLachlan et al., 2007).

Nowadays, Graves disease diagnosis is based on the usual clinical manifestations of hyperthyroidism, diffuse enlargement of thyroid in ultrasound, and the the positive expression of thyrotropin receptor antibody, thyroglobulin antibody, and thyroid peroxidase antibody. However, in the early stage of Hashimotos, there may also be the clinical manifestation of hyperthyroidism, positive thyroglobulin antibody, and thyroid peroxidase antibody. For example, 70% of GD patients have positive TPOAb and TGAb. Similarly, Thyrotropin receptor antibody is also positive in a few Hashimotos patients (Cui, et al., (2019), Effraimidis, G. and Wiersinga, W., (2016)). Therefore, the therapist faced difficulty distinguishing graves and Hashimoto's disease with ordinary clinical symptoms and positive antibodies. Nowadays, the usual test in laboratory is serum thyroid-stimulating antibody determination and thyroid-stimulating blocking antibody specification. thyroid-stimulating antibody is dominant in GD patients was generally found. Thyroid-stimulating blocking antibody domination is an incidence of hypothyroidism is raised (Diana, et al., 2017, Takasu, and

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Matsushita, 2012). Anyway, the detection approach of Thyroid-stimulating blocking antibody and Thyroid-stimulating antibody are basically utilized for scientific research, on the other hand excluded for clinical detection for the diagnosis and subsequent curing of the GD and HT. till now, no effective differentiation method. That's why, the model presents aid for clinicians to specify the indicators as making a precise diagnosis, giving a reference for the clinical diagnosis and identification of hyperthyroidism to effectively save existing medical resources and reduce the economic burden on patients (Cui et al., (2019).

II. MATERIALS AND METHODS

A total of 40 GD and HT patients and 20 normal healthy volunteer controls were enrolled in this work. Patients age and control people enrolled through this study extent from 20-71 years old and contain 20 GD (50%) and 20 HT (50%) out of forty patients. Samples were collected who were diagnosed with GD and HT lesion and attended the Private Hospital in Baghdad Governorate during the period from January 2022 to March 2022. Many diagnosis methods are applied for Patients either clinical examination of nuclear thyroid scan or thyroid ultrasound in addition to biochemical tests before treatment to identify the etiology of hyperthyroidism and hypothyroidism, and the size of the thyroid gland.

2.2 Components of Novel Thyroid-stimulating hormone antibodies (TSHRAb) Eliza kit used in this study are indicated in table (1-1)

Table (1-1):-TSHRAb Elisa Kit Components (De Medi Tech company, Germany)

Component	**Volume (ml)**
Start buffer	10
Calibrators 1-4	1,2,8 and 40 u/L, 4×1.0
Control 1 Negative control Control 2 Positive control	each with 1.0
TSH Biotin	3 vials, each with 4.5
TSH-Biotin Reconstitution Buffer	15
SA-POD 20x Streptavidin peroxidase (SA-POD)	0.75, diluted 1 in 20 with diluent for SA-POD
DIL SA-POD Diluent for SA-POD	15
SUB TMB Peroxidase Substrate (TMB)	15
WASH SOLN 10X, Concentrated wash Solution	100, dilute 1 liter with pure water before use
STOP SOLN Stop solution	10

Novel protocol of serum Thyroid-stimulating hormone receptor antigen according to (Felig, 1987) Thyroid-stimulating hormone receptor antibody was detected, with the following modifications:

Stage 1: preparation of 1st plate for diagnosis of Graves disease: test samples and Reagents left for at least 30 minutes to stand at room temperature (25oC) before use. A repeating Eppendorf-type pipette is preferable for steps 1, 5, 8, 10, and 11. Duplicate locations are strongly preferred for test sera, calibrators, and controls.

A plastic kit was used and 100 microliters of TSHR solution (conc.: 10microgram/dl in 100ml) were added to all wells, then incubated for 24 hours, and washed once, as each well contents were removed, rinsed once with 300µl of washing buffer (1X).

10x of blocking reagent was prepared and added to all wells, then washed once, as each well contents were removed, and rinsed once with 300µl of washing buffer (1X).

The plate was stored.

AlAn aliquotf 75µl of start buffer was added to each well, and the last well was left empty as a blank.

An aliquot of 75µL of test sera, calibrators, and controls was added to respective wells (start with the 40 u/L calibrators and descend the plate to the negative control and then test sera), leaving the last well as a blank.

The frame was covered for coupled hours at room temperature, then discarded samples by briskly inverting the frame of wells over a suitable receptacle. The inverted wells were tapped softly on a clean, dry, absorbent surface to eliminate extra wash solution (only necessary if washing plate by hand), the wells were washed once with a diluted wash in a solution.

Aliquot of 100µL of reconstituted TSHR-Biotin was pipetted into each well (except blank), and the frame, was covered and incubated at room temperature for 25 minutes.

Aliquot of 100µL of diluted streptavidin peroxidase (SA-POD) was added to each well (except blank), then the frame was covered and incubated at room temperature for 20 minutes.

After that, the wells were washed twice with diluted wash solution, then 100µL of SUB 3,3′,5,5′-Tetramethylbenzidine (TMB) was added to each well (including blank) and incubated in the dark at room temperature for 30 minutes.

An aliquott of 50µL stop solution was then added to each well (including blank), and the frame after that was covered, and shaken for about 5 seconds on an ELISA plate shaker. Substrate incubations were goaled as the same for every well.

450 nm wavelength absorbance was recorded for 15 minutes by using an ELISA plate reader, against blanked the well-containing 100µL of SUB TMB and 50µL with only stop solution.

Stage two: preparation for diagnosis of Hashimoto's disease

1. Incubation:

- coating of TSHR on a plastic ELISA plate by adding 100µl of TSHR (10µg/dl in 100 buffer) and incubating for one day.

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- coating reaction then Blocking by the black receptor.

2. Detection:

- for every case-control group Aliquot of 100µl serum was added and incubated for a half-hour, after that the plate was washed twice with diluted buffer.
- Aliquot of 50µl of TSHR was added to the well and incubated for fifteen minutes, and then doubled-washed with diluted buffer.
- synchronize receptor was added and incubated for a half-hour, then three times washing with diluted buffer was done.
- Aliquot of 50µl of TMB was added into each well (with blank) and incubated for 15 minutes, then a 50µl stop solution was added.
- Absorbance at 450 nm was recorded within 15 minutes by using an ELISA plate reader.

2.5 Statistical analysis

The mean of ±SD was utilized to express the result. The Statistical Analysis System-SPSS (2019) program was utilized for the statical analysis. Data from parameters within the study were analyzed for variations using a t-test. Variations have values of $P \leq 0.05$ were deemed to be statistically worthy. Otherwise, the one of values of $P \leq 0.01$ were considered to be statistically highly honorable.

III. RESULTS AND DISCUSSION

For five decades, improvements in the specificity and sensitivity of thyroid tests have gradually impacted the clinical strategies for detecting and treating thyroid disease. For decades, technological advances in immunometric and radioimmunoassay assay procedures have progressively developed the sensitivity and specificity of the methods (Joshi, 2011). The TSHRAb measurement is a specific and sensitive tool for accurate and rapid and differential diagnosis of Graves hyperthyroidism (Kahaly et al., 2018). Thyrotropin receptor antibody which stimulates the TSH receptor plays a huge role in the development and occurrence of graves disease.

Therefore, this study aimed to identify a novel fast, and low-cost diagnosis kit to differentiate between Graves and Hashimoto's disease depending on the Elisa technique principle. Results (figures (1-1)) showed that there are two slides were used for this purpose, the first slide used to diagnose Graves disease patients, and the second one used to diagnose Hashimoto's disease patients according to the linkage between the patient's antibodies and TSHRAb or antigen.

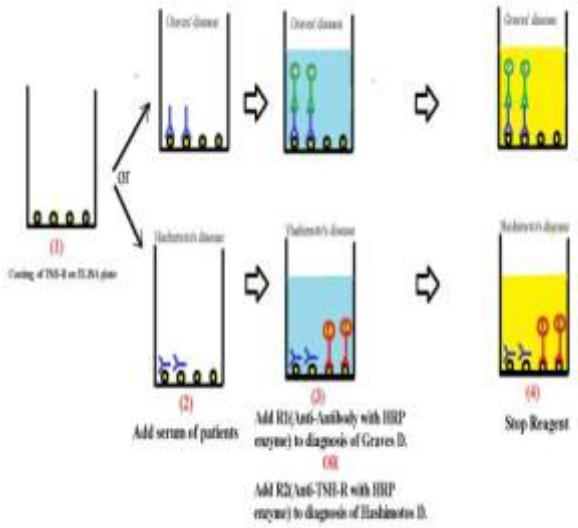

Figure (1-1): Principle of diagnosis of Graves and Hashimoto's disease patients

Methods for measuring Thyrotropin receptor antibody are even more varied, but this method depends on qualitative estimation using the indirect Elisa technique, two classes of TSHRAb can be associated with autoimmune thyroid disorders (1) thyroid-stimulating autoantibodies that cause Graves' hyperthyroidism, and (2) thyroid stimulation-blocking antibodies (TBAb) which block receptor binding of TSH. Each class of Thyrotropin receptor antibody was detected alone in Graves' disease and Hashimoto's thyroiditis. The relative concentrations of the two classes of Thyrotropin receptor antibodies may modulate the severity of Graves' hyperthyroidism and may change in response to therapy.

Tables (1-2) indicate a significant increase in cut-off value in the GD patients group than in healthy control. The principle for this results is the binding of TSHR coated on the Elisa wells with TSHRAb from patients serum in Fab region of patients antibody, which help stimulate hyperthyroidism, then adding R1 anti-anti-TSHRAb with HRP enzyme to the diagnosis of Graves disease. The normal cut-off value in this kit was (0.8), the maximum normal value among the results. Results also showed that there is nonsignificant differences in TSHR-Ab level in GD patients when evaluated using the newly invented kit and the commercially used kit.

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Table (1-2): Level of serum thyroid-stimulating hormone receptor antibody (TSHR-Ab) binding to Fab region in GD serum compared to healthy control

No.	TSHR-Ab (Healthy samples)	TSHR-Ab (GD disease patients using the new kit)	P-value (GD Between healthy control and patient samples)	TSHR-Ab (GD disease patients using a commercial kit)	P-value (GD Between new kit and commercial kit)
1.	0.1	0.024	≤0.0231 **	0.022	NS
2.	0.5	0.549	NS	0.429	NS
3.	0.5	1.446	≤0.0041 **	0.978	NS
4.	0.4	1.121	≤0.071 **	0.992	NS
5.	0.8	0.035	≤0.0001 **	0.102	NS
6.	0.3	1.693	≤0.0001 **	1.361	NS
7.	0.4	1.246	≤0.0001 **	1.257	NS
mean	0.428	0.927	≤0.0621 **	0.734	NS

Different letters in raw means a significant difference
** mean highly significant (P≤0.01), NS: mean non-significant

Fröhlich and Wahl (2017) indicated the prevalence of anti-TSHR antibodies is very common in graves disease patients but relatively rare in patients with Hashimoto's disease, while the prevalence of anti-thyroperoxidase and anti-thyroglobulin antibody is increased in patients with graves disease and Hashimotos disease. This may suggest that anti-TSHR antibodies are produced under more specific cases than the other antibodies.

Furthermore, Walsh, 2016 reported that positive TSH-receptor antibodies indicate graves' disease, therefore this study intends to analyze the dynamic changes of serum thyroid-stimulating hormone receptor antibody in Graves' disease before radiotherapy.

Results indicated in the table (1-3) showed that there is a significant increase in cut-off value in HT disease patients compared with healthy control in the second slide. The principle for this results is the binding of TSHR that coated on the Elisa wells with thyroid-stimulating hormone receptor antibody from patient's serum in Fc region of patients antibody which helps the blockage of thyroid hormone secretion, then adding R2, anti-anti-TSHRAb with HRP enzyme to a diagnosis of Hashimoto's disease.

Table (2-3): Level of serum thyroid-stimulating hormone receptor antibody (TSHR-Ab) binding to Fc region in HT disease

No.	Healthy samples	Hashimoto's patients using the new kit	P-value	Hashimoto's patients using a commercial kit	P-value (Between TSHR-Ab using the new kit and TSHR-Ab commercial kit)
1.	0.1	0.07	≤0.0631 *	0.09	NS

2.	0.5	0.031	≤0.0041**	0.061	NS
3.	0.5	0.12	≤0.0041 **	0.93	NS
4.	0.4	0.08	≤0.0001 **	0.09	NS
5.	0.8	0.02	≤0.0001 **	0.21	NS
6.	0.3	0.05	≤0.0011 **	0.065	NS
7.	0.4	0.04	≤0.0001 **	0.038	NS
mean	0.428	0.058	≤0.0001 **	0.212	NS

Different letters in raw means a significant difference
** mean highly significant (P≤0.01), * means significant (P≤0.05)
Results in tables (1-2) and (1-3) indicated that there is a significant difference in cut-off value in graves disease and HT disease patients depending on Stimulating antibodies to TSH receptor, causing increased or decreased thyroid hormone secretion. Results also showed that there is nonsignificant differences in TSHR-Ab level in HT patients when evaluated using the newly invented kit and the commercially used kit.
Dong et al. (2017) showed non-significant differences in serum TSHR-Ab levels in GD patients before treatment with RAI-131 treatment respectively. It also showed that serum TSHR-Ab levels showed dynamic changes in level which increased at three months, increased to peak at six months, and diminished to baseline at twelve months. While this novel kit enables the diagnosis of GD patients at any time of medication, monitoring the level of binding of TSHR-Ab could be useful to assess the prognosis of treatment of radiotherapy in Graves' disease (Iddah and Macharia, 2013).

IV. CONCLUSION

TSHR-Ab kit is a super diagnostic kit to identify the difference between Hashimoto's or Graves disease without the high-cost TSHR kit. The prognosis of autoimmune thyroid disease will support therapeutic purposes and the titer will identify the level of severity of the disease.

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V. REFERENCE:

[1]. Ross D., Burch H., Cooper D., Greenlee M., Laurberg P., and Maia A., et al. (2016). American Thyroid Association guidelines for diagnosis and Management of Hyperthyroidism and Other Causes of thyrotoxicosis. Thyroid. 2016; 26(10):1343–421.

[2]. Fröhlich E., and Wahl R., (2017). Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and Extra-Thyroidal Diseases. Front. Immunol. 8:Pp521-531.

[3]. Michelle N., Jeffrey A., (2021). A Grave Turn on Hashimoto's Thyroiditis - A Case Series on Four Patients With Autoimmune Hypothyroidism that Converted to Grave's Disease, Journal of the Endocrine Society. V (5):1, Pp: 913–914, https://doi.org/10.1210/jendso/bvab048.1865

[4]. Joshi., R., (2011). Laboratory evaluation of thyroid function. The Journal of the Association of Physicians of India. VOL. 59. Pp:14-20.

[5]. McLachlan S., and Rapoport B., (2017). Thyrotropin-blocking autoantibodies and thyroid-stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. Thyroid; 23:Pp:14-24.

[6]. Cui et al. (2019). Establishment of clinical diagnosis model of Graves' disease and Hashimoto's thyroiditis. J. Transl Med, Pp 17:11.

[7]. Effraimidis, G. and Wilmar M., (2016). Mechanisms in endocrinology: Autoimmune thyroid disease: old and new players. European Journal of Endocrinology 170(6), Pp:14-27.

[8]. Diana T., Krause J., Olivo P., König J., Kanitz M., and Decallonne B., et al., (2017). Prevalence and clinical relevance of thyroid-stimulating hormone receptor-blocking antibodies in autoim1mune thyroid disease. Clin Exp Immunol.;189(3):Pp: 304–9.

[9]. Takasu N, and Matsushita M. (2012). Changes of TSH-stimulation blocking antibody (Thyroid-stimulating blocking antibody) and thyroid-stimulating antibody (Thyroid-stimulating antibody) over 10 years in 34 Thyroid-stimulating blocking antibody-positiv patients with hypothyroidism and 98 Thyroid-stimulating antibody-positive Graves' patients with hyperthyroidism: reevaluation of Thyroid-stimulating blocking antibody and Thyroid-stimulating antibody in

TSH-receptor-antibody (Thyrotropin receptor antibody)-positive patients. J Thyroid Res. 18-2176.

[10]. Felig P., (1987). Endocrinology and metabolism. New York, 389.

[11]. Kahaly et al., (2018). 2018 European Thyroid Association Guideline for the Management of Graves' Hyperthyroidism. Eur Thyroid J.; 7: Pp:167–186. DOI: 10.1159/000490384.

[12]. Fröhlich E and Wahl R., (2017). Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and Extra-Thyroidal Diseases. Front. Immunol. 8:521.

[13]. Walsh, J., Rothacker, K., Brown, S., Hadlow, N., Wardrop, R. (2016). Reconciling the log-linear and non-log-linear nature of the TSH-free T4 relationship: intra-individual analysis of a large population, The Journal of Clinical Endocrinology and Metabolism, 101(3): 1151–1158.

[14]. Dong, X,. Liu, F., Wang, Y. Xu, C., Liang, W. Du, Gao, G., (2017). Dynamic changes of Thyrotropin receptor antibody and TPOAb after radioiodine therapy in Graves' disease. Acta Endocrinologica (Buc), XIII: 1; Pp. 72-76.

[15]. Iddah and Macharia, 2013. Autoimmune Thyroid Disorders. ISRN Endocrinology. 509764, 9.

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