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Anti-TRA-Ab, Anti-TPO-Ab, and FT3 as a Biochemical Panel for Differential Diagnosis of Graves' Disease from Hyperthyroidism

KEYWORDS: Anti-TRA-Ab, Anti-TPO-Ab, FT3, Graves' disease

Dr. Ghada A.Elfadil	Isam Eddin M Eltahir	Dr. Abdelgadir A. Elmugadam
College of Applied Medical Science, Taibah University. KSA	College of Medical Laboratory Science, Sudan University of Science & Technology	College of Medical Laboratory Science, Sudan University of Science & Technology

ABSTRACT *High titers of antibodies to anti thyrotropin receptor (TRA-Ab), and thyroperoxidase (TPO-Ab) antibodies are the hallmarks of human autoimmune thyroid diseases. The aim of this study was to determine the titers of these antibodies in Sudanese Patients (mean age 33.22±10.04, range 19-59 years) with hyperthyroidism and graves' disease. This study was done in order to investigate any correlation regarding clinical manifestations that may be unique to patients. One hundred Sudanese patients with hyperthyroidism(50with hyperthyroidism and clinically diagnosed with graves' disease, 50 with hyperthyroidism and clinically diagnosed without graves' disease), and fifty apparently healthy as control group , age and sex were matched. Study done in Endocrinology Outpatient Clinic at Omdurman Teaching Hospital, Khartoum state, Sudan, during the period June 2011 to June 2013. Thyroid function test (FT3, FT4, and TSH) was determined by using fully automated chemical analyzer (ROCH ELECSYS) with COBAS kits, and anti thyroperoxidase antibodies, and anti thyrotropin receptor antibodies were determined by ELISA technique with Euro immune Kits. Analysis of the data was performed using the Chi-square test with P< 0.05 considered significant. Study showed that there were significantly high serum FT3, anti TRA, TPO antibodies among the Sudanese patients with Graves disease compared with patients with hyperthyroidism. 90% of patients with graves' disease had positive TRA antibodies, and 16% had positive TPO antibodies.*

Conclusion: In conclusion, the parameters, FT3, anti-TRA- Ab, and anti-TPO-Ab can be used as a biochemical panel for differential diagnosis of Graves' disease from hyperthyroidism.

Introduction:
Graves' disease (GD) is an autoimmune disorder characterized in its typical presentation by the unique association of thyrotoxicosis, goiter and ophthalmopathy [(1)]. The exact etiology of GD is overall still unknown. However, the majority of investigators share the concept that GD is a multifactorial disease caused by a complex interaction between genetic and environmental factors that lead to the loss of immune tolerance to thyroid antigens, and therefore to the initiation of an immune reaction against the thyroid [(2).]

GD is one of the most frequent diseases among autoimmune disorders, with an annual incidence of approximately 14 per 100,000 [(3)]. In iodine sufficient areas, it accounts for 70-80% of all cases of thyrotoxicosis [(4)]. As in most autoimmune diseases, GD is more frequent in women than in men, with a ratio of approximately 5/1. GD can be observed at any age, including childhood, although its incidence peaks between the fifth and sixth decades [(5)].

Ethnical differences in the incidence of GD have not been investigated consistently, although there seems to be a higher prevalence of the disease in Caucasians and Asians than in Africans [(6)].

Graves (GD) disease is caused by circulating antibodies that mimic the action of the thyroid stimulating hormone (TSH), namely binding to and activating its receptor (TSHR), resulting in increased synthesis and release of thyroid hormones (hyperthyroidism) and hypertrophy of thyroid follicular cells (goiter). Ophthalmopathy, the most common extra thyroidal feature of GD, is clinically present in about 50% of patients, and, although its pathogenesis remains to be completely elucidated, it is believed to be due to an autoimmune reaction against antigens shared by the thyroid and orbital tissues, among which the TSHR is the most reasonable candidate [(1)]. Thyrotropin receptor antibody (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 [(7)].

Anti-thyroid peroxidase antibody (anti-TPO) is important in diagnosing autoimmune thyroid disease and for judging treatment efficacy [(8)]. Anti-TPO is also found in sera of about 10% of normal adults, with an increasing prevalence (up to 30%) in older adults [(9)]. More patients with thyroiditis have high serum anti-TPO than anti-Thyroglobin antibody concentrations[(10)].

Materials and methods:
One hundred Sudanese patients with hyperthyroidism (50with hyperthyroidism and clinically diagnosed with graves' disease, 50 with hyperthyroidism and clinically without graves' disease), and fifty apparently healthy as control group , age and sex were matched.

Study done in Endocrinology Outpatient Clinic at Omdurman Teaching Hospital, Khartoum state, Sudan, during the period June 2011 to June 2013. An informed consent procedure was approved by the local medical authority, and all patients were informed about the purpose of the study. Venous blood samples(5mL)were taken from each participant by using disposable syringe. The blood samples allowed to clot for 20 minutes at room temperature and then serum was obtained after centrifugation at 300rpms. Thyroid function test (FT3, FT4, and TSH) was determined by using fully automated chemical analyzer (ROCH ELECSYS) and COBAS kits, and, anti Thyroperoxidase antibodies (TPO-Ab), and anti thyrotropin receptor antibodies (TRA-Ab) were determined by ELISA technique with Euro immune Kits.

Results:
The mean age of patients with hyperthyroidism without graves' disease was (33.22±10.04 years), while the mean age of patient with graves' disease was 34.82±11.13 years, and control group was (34.82±11.13)years, p. value 0.394. The

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concentration of both thyroid antibodies were measured by quantitative ELISA, then transformed into negative (less than14.5U/L), Equivocal (14.5-35.5 U/L), positive(more than 35.5 U/L) for anti-TPO, and negative (less than1 U/L), Equivocal (1-2 U/L), positive(more than 2 U/L) for anti-TRA.

Table1: Comparison between means of age, TSH, FT3, FT4,TPO, TRA in Sudanese patients with hyperthyroidism and with Graves disease compared with control group.

	Means ±SD	P value
Age (year) hyperthyroidism without graves	33.22±10.04	0.394
Graves	34.82±11.13	0.459
control	34.82±11.13	
TSH U/L Hyperthyroidism without graves	0.028±0.035	0.00*
Graves	0.02±0.016	0.00*
Control	3.28±0.9	
FT4 ng/dL hyperthyroidism without graves	27.30±28.8	0.00*
Graves disease	32.6156±36.46	0.00*
Control	2.86±0.45	
FT3 Pg/mL hyperthyroidism without graves	14.30±9.50	0.00*
Graves disease	21.4±20.6	0.00*
Control	1.43±0.3	
TPO Pg/mL hyperthyroidism without graves	5.0480±1.92	0.41
Graves disease	21.7800±13.88	0.00*
Control	5.04±1.90	
TRA U/mL hyperthyroidism without graves	1.377±0.099	0.00*
Graves disease	9.3242±7.71	0.00*
Control	0.7±0.3	

***Independent sample T.test was used for comparison. P. value considered significant at level <0.05**

Table 2: Comparison between means of TSH, FT3, FT4, TPO, TRA in Sudanese patients with graves' disease and hyperthyroidism without Graves disease .

Study group	Means ±SD	P value
TSH U/L Graves disease	0.024±0.028	0.117
Hyperthyrodism	0.028±0.035	
FT4 ng/dL Graves disease	32.6156±36.46	0.421
Hyperthyroidism	27.30±28.8	
F T3 pg/mL Graves disease	21.41±20.6	0.03*
Hyperthyroidism	14.30±9.50	
TPO Pg/mL Graves disease	21.7800±13.88	0.00*
Hyperthyrodism	5.0480±1.92	
TRA U/mL Graves disease	9.32±7.7	0.00*
Hyperthyrodism	1.377±0.099	

***Independent sample T.test was used for comparison. P value considered significant at level <0.05**

Table 3: distributions of thyroid antibodies in Sudanese patients with Graves disease compared with control .

	Graves disease	Control	Hyperthyroidism
TPO-Ab Positive	16% (n=8)	0%	0%
Equivocal	54% (n=27)	0%	0%
Negative	30% (n=15)	50%	100%
TRA-Ab Positive	90% (n=45)	2% (n=1)	0%
Equivocal	0 %	26%(n=13)	100%
Negative	10% (n=5)	72% (n=36)	0%

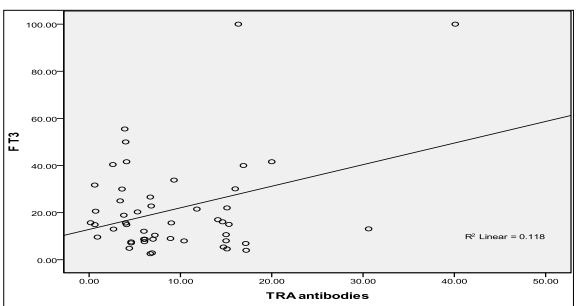

Fig 1: shows correlation between serum titter of TRA antibodies and serum FT3 among Sudanese patients with Graves disease. Significant positive correlation (p= 0.0115, r=0.34)

Discussion:

Over the past five decades, improvements in the sensitivity and specificity of thyroid test methodologies have dramatically impacted the clinical strategies for detecting and treating thyroid disorders [11].

The mean age at onset of hyperthyroidism disease was 34.8± 11.13 years with range 18-59 years ,This finding is in agreement with those reported by Biassoni et al [12].

In the patients with graves' disease study showed there were significant increase in mean of FT3, FT4, and significant decrease in mean of TSH compared with control group,This indicate that the laboratory hallmark of graves is the finding elevated levels of serum thyroxine (T4) and triiodothyronine (T3), associated with undetectable serum TSH. Because total T4 and T3 measurements are influenced by multiple conditions affecting serum thyroxine-binding globulin (TBG) [13], free thyroid hormone (free T4 (FT4) and free T3 (FT3) measurement, is nowadays considered the gold standard for the diagnosis of thyrotoxicosis.TSH is the initial screening test in the evaluation of a suspected thyrotoxicosis, but it is strongly recommended to add measurement of FT4 to improve diagnostic accuracy[14].

Antibodies against the TSH receptor (TRAbs) are pathognomonic for graves' disease, our study showed significant increase in mean of TRA in patients with graves when compared with both patients with hyperthyroidism and control group. TRA was detected in 90% of patients with graves' disease, and undetected among control group in this study. They are detectable in the serum of about 98% of untreated GD patients using a second generation assay [15]and in an even higher proportion of patients using a third generation assay [16]. TRA-Ab measurement is a very valuable tool in the differential diagnosis between GD and thyrotoxicosis due to other causes, and, the detection of TRA- Abs rules out other causes of thyrotoxicosis [17]. However,TRA- Ab measurement is required when the clinical picture is not clear [18] .

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Serum anti-thyroid peroxidase (TPO) was significant increase in patients with graves, when compared with both hyperthyroidism and control group, 16% of patients with graves had a positive in this study. Other study in Sudan shows 66.7% of the graves patient have positive TPO[18].Serum anti-thyroid peroxidase antibodies (TPO), although detectable on the majority of GD patients, is generally not useful for GD diagnosis [19]. Our recommendation, importance of screening for anti TRA- Ab, TPO-Ab in non-autoimmune thyroid disorders. TRA antibodies is best laboratory maker in diagnoses of Graves disease, it can make the diagnosis almost unmistakable.

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