Original Article

Thyroid Receptor Antibodies and Thyroid Peroxidase Antibodies in a Sample Thyrotoxic Patients: A Cross-Sectional Study

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Abstract

Background: Thyrotoxicosis is a clinical status due to hypersecretion of thyroid hormones by diffuse goiter (Grave's disease [GD]), multinodular goiter, single toxic adenoma, and pituitary adenoma secreting thyroid-stimulating hormone (TSH) rarely. GD: It is diffuse toxic goiter (GD) or (Basedow disease) it is a triad of: Diffuse toxic goiter, hyperthyroidism, and exophthalmos (proptosis). **Aims:** 1. Positivity of TRAb and TPO in thyrotoxic subjects. 2. Correlation of the titer of these antibodies with the clinical status of the patients. 3. Correlation between TRAb and TPO titer. 4. To find out if TPO titer on enrollment has any correlation with the clinical status of the patients. **Methods:** A cross-sectional study conducted in the National Diabetes Center–Mustansiriyah University in the period from November 2021 to April 2022 where 93 patients with GD are enrolled to check their thyroid status and check some biochemical variables in their sera as thyrotropin receptor antibody (TRAB), thyroid peroxidase (TPO) antibody, TSH, and free thyroxine (FT4). 44.6% are women and 35.7% are men, at the time of recreuitment 49.4% are toxic while the remaining 58.6% are euthyroid being on anti thyroid drugs. 87 persons are recruited as normal euthyroid, they are sex and age-matched, the control TRAb were negative. **Results:** GD patients are as follows: 54 (58.06%) euthyroid and 39 (41.94%) toxic at the time of recruitment. Eighty-two percent of toxic patients have goiter and 74.07% of euthyroid GD patients have goiter. Ophthalmopathy is found in (64.1% of toxic GD patients and 42.59% of euthyroid GD patients. TPO median in the control, toxic, and euthyroid GD patients is (22.76%), (75%) and (63.5%) (highest among toxic GD patients) ($P < 0.001$). TSH in the control group has a mean of (2.18 ± 1.72) and a median of (1.89). The TRAb is the highest in toxic GD patients, followed by euthyroid GD patients and the least in the control, its mean is (9.98 ± 8.42), (7.24 ± 7.8) and (0.93 ± 0.15), respectively. It is recommended to conduct a longitudinal study in which patients with GD are checked at variables times in the course of illness (remission and relapse) studying these biochemical and immunological markers in these variable states of thyroid function. **Conclusion:** Ninety-three thyrotoxic patients, 39 are toxic and 54 are euthyroid on arrival. Eye sings are more in toxic patients, goiter and eye signs are predictor of GD, TRAb is the highest among toxic patients, TPO are higher among GD patients versus the control.

Keywords: Grave's disease, thyroid peroxidase antibodies, thyroid-stimulating hormone-receptor antibodies

Introduction

Grave's disease (GD) was discovered in the 19th century by Von Basedow and Robert Graves. Finally, the pathogenic importance of thyroid-stimulating auto-antibodies was discovered by Adamas and Purves. The central elements of (GD) are the circulating thyroid-stimulating hormone (TSH): receptor antibodies thyrotropin receptor antibody (TRAb). Eighty percent of thyrotoxicosis is caused by GD. Fifty percent of patients with GD have goiter detected by ultrasound. Infiltrative Grave's ophthalmopathy (GO) is the very important sign. The remaining signs include: loss of weight, hyperphagia, profuse sweating, loose bowel motions, palpitations, and heat intolerance.[1,2] Thyroid-stimulating antibody receptor (TRAb) is directed to TSH receptors to stimulate them when they are expected to cause hypermetabolic state in cases of toxic GD patients.[3] Pathologically, the gland is infiltrated by lymphocytes in a nonhomogeneous distribution without creating follicles. The degree of infiltration is reduced by using anti-thyroid drugs.

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The abundant lymphocytes include Th1, Th2, and CD25+, whereas B-lymphocytes are scarce,[4] The TSH receptors have transmembrane domain with a large extracellular and small intercellular domain.[5-8]

Patients and Methods

Ninety-three patients with thyrotoxicosis are enrolled in the study during the period from November 2021 to April 2022. Their age range 17–71 years (41.1 ± 15.11). They are known to be toxic clinically and biochemically at the time of recruitment 39 patients (41.94%) while the remaining patients 54 (58.06%) patients are euthyroid being on anti-thyroid drugs such as methimazole, neomercazole, or propylthiouracil. They are examined by the physician to check their eligibility to be recruited, preceded by detailed history and meticulous physical examination to look for relevant symptoms and signs of hyperthyroidism. Twenty-nine (44.6%) of enrolled women and 10 (35.7%) of the enrolled men are toxic. The control group is healthy counterparts composed of 87 persons their age ranges from 20 to 62 years (43.33 ± 11.71); they are 59 women and 28 men. The controls are sex- and age-matched healthy patients for whom thyroid biochemical profile is normal plus negative TRAb antibodies.

Both groups of patients and healthy controls are given enough time to discuss their acceptance to participate in the study, this verbal consent was taken from both groups.

The endocrinologist predicts their TSH receptor antibodies (TRAb) to be expected either positive or negative on the basis of presence of goiter and or proptosis as: Lid retraction, lid lag, staring eyes and protrusion of the globe beyond the lateral bony orbit.[9]

Blood samples were taken from every participant to check thyroid hormones TRAb antibodies, thyroid peroxidase antibodies (Anti-TPO) antibodies, and free thyroxine (FT4) The markers are measured then statistical studies were conducted to compare these variables between the group of thyrotoxic patients and the controls and between the two subgroups of patients at the time of sampling when they are toxic.

The presence of goiter and proptosis are studied to find out their impact on the treating physician to predict GD. By using logistic regression model, the sensitivity and specificity of the studied variables (TRAb, TSH, TPO, and FT4) by using receiver-operating characteristic curve and the area under the curve were shown to reflect the sensitivity and specificity of each variable by using (Statistical Packages for Social Sciences-version 25) (IBM Corp., Armonk, NY).

Exclusion criteria

Patients who have been treated by thyroidectomy and/or radioiodine therapy, pregnant ladies, toxic multinodular goiter, toxic with single nodule, and those with thyroiditis were excluded from the study.

Ethical considerations

The ethical committee in the College of Medicine/ Mustansyriah University had approved the study, and for each patient, a verbal consent has been taken to participate in the study.

Blood collection

Ten milliliters of venous blood were withdrawn from the veins of the hand dorsum or antecubital fossa by disposable 10 mL syringe to be collected in a clot 2 activator tube and left to clot over a period of 20 min. Followed by centrifugation at 1000–2000 RPM for 15 min. The collected sera were used to measure TRAb, TPO, FT4, and TSH instantaneously.

Determination of anti-thyroid-stimulating hormone receptor, thyroid-stimulating hormone, anti-thyroid peroxidase, free thyroxine, and thyroxine

The above-mentioned markers are measured by using Roche e411 (Germany).

Electrochemiluminescence

The principle of (electrochemiluminescence) depends on the detection of photons generated by chemiluminescent material attached to the antibody that acts as sensor for the antigen of interest to be measured.

Results

The enrolled GD patients are 93, they are 54 euthyroid (58.06%) and 39 toxic (41.94%) at the time of recruitment, woman with GD are 36 euthyroid and 29 toxic patients. Regarding the presence or absence of goiter, 77.42% of patients had goiter, whereas the remaining 22.58% had no goiter thus representing 72 and 21 patients with GD, respectively.

Forty-eight (51.61%) had positive proptosis representing 25 toxic patients on arrival and 23 euthyroid patients on arrival, while the remaining 45 patients had no proptosis of whom 14 are toxic and 31 are euthyroid on arrival.

Positive TRAb in toxic was detected in 32 patients out of 39 toxic GD patients (82.05%), while in euthyroid GD patients, only 35 out of 54 were found to have positive TRAb (64.81%).

Subclassification of TRAb-positive patients revealed that 32 patients are toxic while the remaining 35 are euthyroid.

Physician prediction is consistent with TRAb positivity in 25 toxic patients (64.1) and 33 euthyroid patients (61.11) so physician prediction of positivity is around 61%–64% irrespective whether the patients yes toxic or euthyroid at the time of recruitment.

Eighty-two (82.0%) of toxic patients have goiter and (74.07%) of euthyroid patients were found to have goiter.

Proptosis was found in (64.1%) of toxic patients and (42.59%) of euthyroid patients, the difference is statistically significant ($P = 0.04$), as shown in Table 1.

In the control group, 87 persons are recruited they are aged matched but definitely vary in TSH level significantly when compared with GD patients ($P < 0.001$).

TPO median in the control, toxic, and euthyroid (GD) patients is 22.76, 75, and 63.5, respectively ($P < 0.01$). The median for TRAb is (0.86) in the control is (8.69) in the toxic, and 4.45 in the euthyroid patients, the difference is highly significant ($P < 0.001$). It was found that TRAb for euthyroid patients is (7.24 ± 7.88), while in the control (0.93 ± 0.15), thus the difference is highly significant ($P < 0.0001$), as shown in Table 2.

Table 2 the value of Eta squared is 0.19 it indicates that the status is responsible for 19% of variation in TRAb as Eta squared measures the effect size for use in analysis of variance which is analogous to R^2 from multiple linear regression. Paired *t*-test was conducted to analyze the set of data deeply; the effect of multiple comparisons was corrected using Tukey HSD *P* value adjustment to reduce family-wise error. The mean TRAB for toxic patients is (9.98 ± 8.53) while for the control is (0.93 ± 0.15), the difference is highly significant ($P < 0.001$).

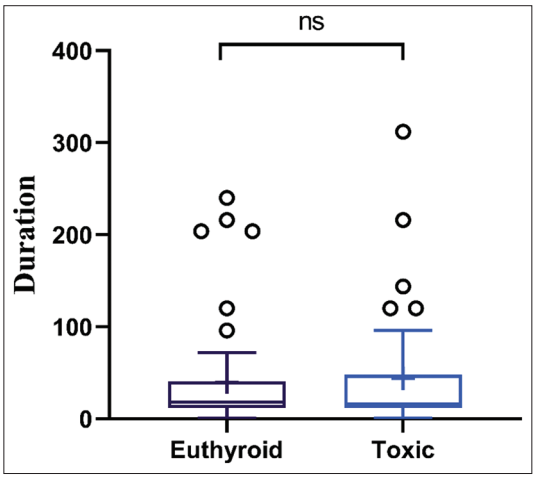

Figure 1: Duration of disease in toxic and euthyroid GD patients in months. GD: Grave's disease

Two tailed Mann–Whitney *U* test did not show statistical difference in the duration of the diseases among toxic and euthyroid patients, as shown in boxplot in Figure 1.

The mean TSH for toxic patients is 0.0928 uIU/ml, while it is 1.0592 uIU/ml for euthyroid GD patients and the difference is statistically highly significant ($P < 0.001$) as shown in Figure 2.

TSH is found to be higher among the enrolled control persons (non-Grave's counterparts) versus the recruited GD patients irrespective of their thyroid status whether they are toxic or metabolically controlled by anti-thyroid drugs, (2.18) uIU/ml versus (0.58) uIU/ml, the difference is statistically significant ($P < 0.001$) which is evident in Figure 3.

FT4 has an average of (35.58 ± 19.85) for toxic GD patients and (14.46 ± 5.22) pmol/L. For euthyroid GD patients, the difference is highly significant using Shapiro–Wilk test ($P < 0.01$) pmol/L as shown in Table 3 and Figure 4.

The mean of thyroxine is (211.769 ± 51.541) nmol/L for toxic GD patients and (106.249 ± 30.7383) nmol/L for euthyroid GD patients and the difference is highly significant as shown in Table 3 and Figures 4 and 5.

Table 4 and Figure 6 show that TPO has a mean of 102.82 IU/ml in GD patients and 77.33 IU/ml in the control, the difference is highly significant from the statistical point of view ($P < 0.001$)

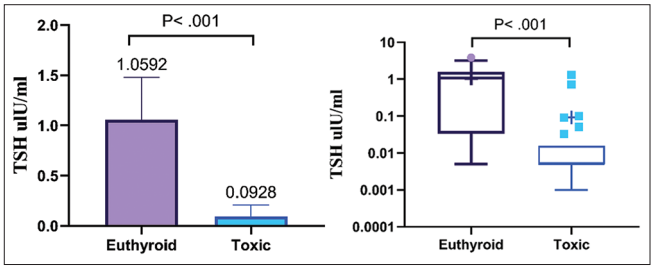

Figure 2: Chart and ranks of TSH by thyroid Status showing significant difference between the groups. TSH: Thyroid stimulating hormone

Table 1: Gender, thyrotropin receptor antibody, goitre, eye signs and prediction of thyrotropin receptor antibody positivity in toxic and euthyroid Grave's disease patients at the time of enrollment

	Variable	Toxic, *n* (%)	Euthyroid, *n* (%)	Total, *n* (%)	χ^2	df	*P*
Gender	Female	29 (74.36)	36 (66.67)	65 (69.89)	0.637	1	0.425
	Male	10 (25.64)	18 (33.33)	28 (30.11)			
TRAb	Positive	32 (82.05)	35 (64.81)	67 (72.04)	3.340	1	0.068
	Negative	7 (17.95)	19 (35.19)	26 (27.96)			
Prediction	Yes	25 (64.10)	33 (61.11)	58 (62.37)	0.0863	1	0.769
	No	14 (35.90)	21 (38.89)	35 (37.63)			
Goitre	G	32 (82.05)	40 (74.07)	72 (77.42)	0.824	1	0.364
	NG	7 (17.95)	14 (25.93)	21 (22.58)			
Proptosis	P	25 (64.10)	23 (42.59)	48 (51.61)	4.195	1	0.041
	NP	14 (35.90)	31 (57.41)	45 (48.39)			

χ^2: Chi-square, df: Degree of freedom, TRAb: Thyrotropin receptor antibody, G: Goitre, NG: No goitre, P: Proptosis, NP: No proptosis

Table 2: Age, thyroid stimulating hormone, thyroid peroxidase, and thyrotropin receptor antibody among Grave's disease patients (both toxic and euthyroid) and the control group of non-Grave's disease counterparts

	Statistic	*n*	1st Q	Median	3rd Q	Mean±SD	SEM	R^2	*F*	Pr>*F*
Age (years)	Control	87	34.00	46.00	52.00	43.33±11.65	1.26	0.02	1.48	0.23
	Toxic	39	24.50	36.00	53.50	38.95±16.14	2.62			
	Euthyroid	54	31.50	41.00	54.25	42.80±13.98	1.92			
TSH (ulU/ml)	Control	87	1.25	1.89	2.54	2.18±1.72	0.19	0.19	20.93	<0.001
	Toxic	39	0.01	0.01	0.01	0.09±0.28	0.06			
	Euthyroid	54	0.04	1.08	1.50	1.06±1.02	0.20			
TPO (IU/ml)	Control	87	18.81	22.76	31.60	65.80±128.65	13.87	0.06	5.28	0.01
	Toxic	39	20.00	75.00	182.00	137.92±174.95	28.38			
	Euthyroid	54	17.00	63.50	188.00	146.87±187.68	25.78			
TRAb (IU/L)	Control	87	0.80	0.86	1.02	0.93±0.15	0.03	0.10	10.27	<0.001
	Toxic	39	2.59	8.69	15.00	9.98±8.42	1.37			
	Euthyroid	54	1.34	4.45	10.38	7.24±7.80	1.07			

The Pr(>*F*) in ANOVA is the probability of observing a difference as large or larger than the one observed, if the null hypothesis were true. R^2=Eta squared, *F*=*F*-test (ANOVA). 1st Q and 3rd Q=Interquartile ranges, SEM: Standard error of the mean, ANOVA: Analysis of variance, SD: Standard deviation, TSH: Thyroid stimulating hormone, TPO: Thyroid peroxidase, TRAb: Thyrotropin receptor antibody

Table 3: Descriptive statistics and means comparisons of the study parameters between toxic and euthyroid patients using a two-tailed independent samples *t*-test

	Euthyroid		Toxic		*t*-tests	
	n	Mean±SD	*n*	Mean±SD	*t*	*P*
FT4 (pmol/L)	53	14.464±5.2226	39	35.575±19.8515	6.478	<0.001
T4 (nmol/L)	17	106.294±30.7383	13	211.769±51.5528	6.541	<0.001

Results for testing the mean differences by clinical thyroid gland status using *t*-tests. SD: Standard deviation, T4: Thyroxine, FT4: Free thyroxine

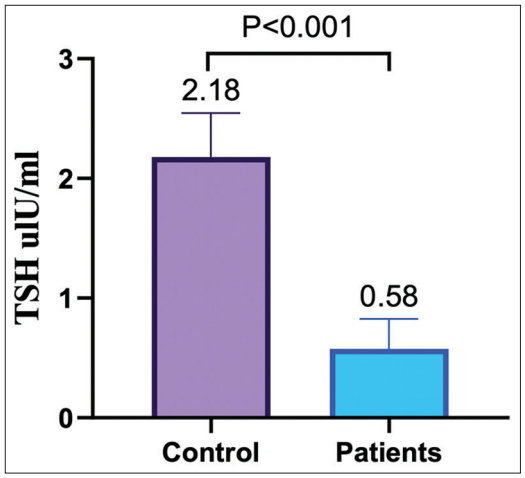

Figure 3: The mean of TSH by levels in the control group and GD patients. TSH: Thyroid stimulating hormone, GD: Grave's disease

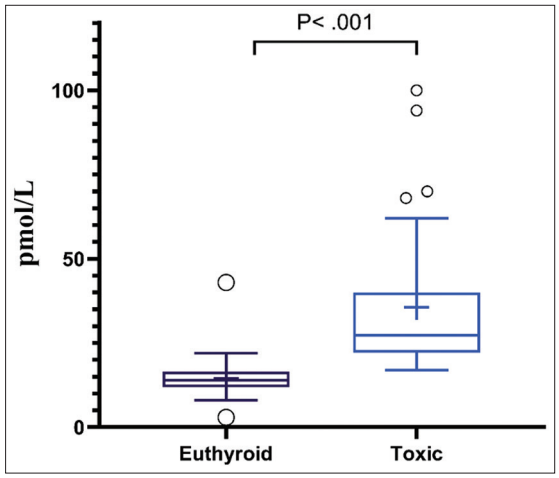

Figure 4: Ranks of FT4 by thyroid Status. FT4: Free thyroxine

while the age is matched between patients and the control group ($P = 0.198$).

The mean TPO has been compared between the two groups by using Mann-Tailed Mann–Whitney *U* test which is a nonprometric test of null hypothesis to compare two dependent samples.

The median TPO is 65.65 IU/ml for the control group and 144.1 IU/ml for GD patients, as shown in Figure 6.

Thyroid receptor antibodies have a mean of (0.93 IU/L) in the control group which is significantly lower than for GD patients (8.39 IU/L), as shown in Figure 7.

Table 5 shows that goiter is a predictor of TRAb positivity by using logistic regression model, it was found that the presence of goiter is very strong sign that persuades the treating physician to predict that patient has GD. The R squared is 0.14 ($P \leq 0.001$). The odds of finding no goiter reduce the chance of predicting Grave's significantly thus in the absence of goiter, the prediction drops by 89% (odds ratio = 0.11).

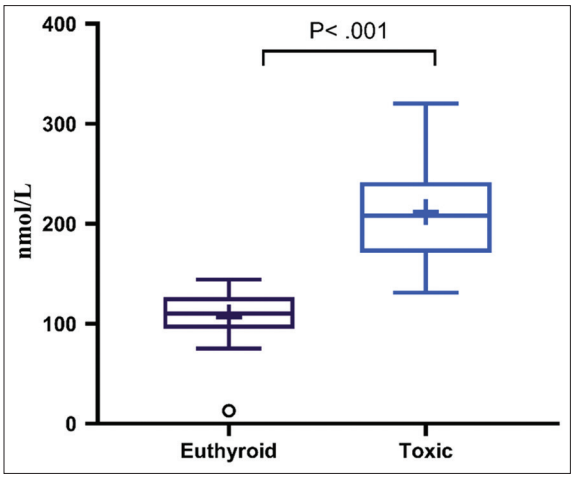

Figure 5: Ranks of TT4 by thyroid. TT4: Total thyroxine

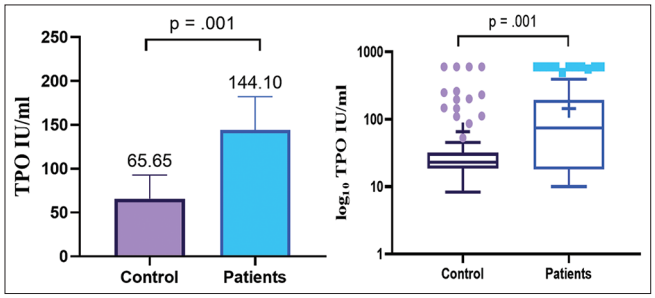

Figure 6: The mean with 95.00% CI Error Bar and rank values of TPO by levels of groups. CI: Confidence interval, TPO: Thyroid peroxidase

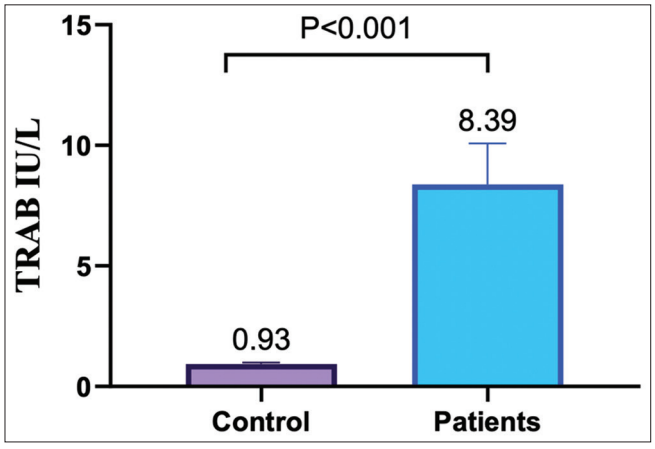

Figure 7: The mean of TRAb by levels of ID with 95.00% CI Error Bars. TRAb: Thyrotropin receptor antibody, CI: Confidence interval

Their effect of detecting proptosis on the treating physician to predict GD is found to be not significant. These findings were found by using regression model, as shown in Table 6.

Discussion

In the current study, 77.42% of patients have clinically visible goiter while the reminder have only goiter detected by sonography. Eighty-two percent of toxic patients have clinically visible goiter while only 74.0% of euthyroid GD patients has clinically detectible goiter.

Fifty-one percent of enrolled patients who are toxic at enrollment have proptosis, while the remaining patients who are euthyroid show no proptosis.

Proptosis is founded in 64.1% of toxic patients and 42.59% of euthyroid patients.

GO occur in 20% of individuals, it may be attributed to other autoimmune diseases directed to the extraocular muscle. GO correlates with the elevation of these antibodies.[10]

TRABs titer is the highest in toxic GD patients followed by euthyroid ones and finally the control. TRAb antibodies help to differentiate GD from other causes of hyperthyroidism. Sensitivity of detection of TRAb is 97.2% and specificity 98% by third generation thyroid binding immunoassay. However, recently, the new commercial platforms have a sensitivity of 100% and specificity of 99%.[11-14]

FT4 and total thyroxine are high among toxic GD patients followed by euthyroid GD patients and finally by the control.

TPO antibodies are the highest among GD patients followed by euthyroid GD patients and the lowest is the control.

The presence or the absence of TPO in GD may have an effect on the clinical causes of the disease. TPO positivity was found more in women (89%) versus men (40%) and the family history

Table 4: Mean thyroid peroxidase level in the control group and Grave's disease patients

Variable	Mean rank		*U*	*Z*	*P*
	Control	Patients			
Age	95.66	85.67	4494.50	−1.29	0.198
TPO	77.33	102.82	2900.00	−3.28	0.001

Ranks of age and TPO by patients and control groups. TPO: Thyroid peroxidase

Table 5: Logistic regression results with goiter predicting (prediction status yes/no)

Variable	*B*	SE	χ^2	*P*	OR	95.00% CI
Intercept	1.04	0.27	15.33	<0.001	-	-
Goitre NG	−2.21	0.58	14.61	<0.001	0.11	0.04-0.34

χ^2 (1)=17.35, P<0.001, McFadden R^2=0.14. SE: Standard error, OR: Odds ratio, CI: Confidence interval, NG: No goiter

Table 6: Logistic regression results with eye predicting (prediction status yes/no)

Variable	*B*	SE	χ^2	*P*	OR	95.00% CI
Intercept	20.57	2559.16	0.00	0.994	-	-
Eye=NP	−21.72	2559.16	0.00	0.993	3.68×10^{-10}	0.00-Inf

χ^2 (1)=73.51, P<0.001, McFadden R^2=0.59. SE: Standard error, OR: Odds ratio, CI: Confidence interval, NP: No proptosis

has its impaction. TPO positivity may increase the chance of relapses after drug discontinuation. TPO positivity in GD is found to be unfavorable as it increases the chance of having severe disease.[15]

TPO positivity may augment autoimmune reaction in genetically susceptible patients.[16-18]

TPO are immunoglobulins G (IgG) G1 (70%), IgG G4 (66.1%), IgG G2 (35.1%), and IgG G3 (19.6%).[18]

TPO contributes to T-cells and the cytokine-mediated thyrocyte apoptosis.[16]

Human T-cells recognize self thyrocytes as foreign antigen, the initiation of the insult of autoimmunity may be induced by external stimuli as infection or trauma, TPO can interact with T-cell clones and TSH external domain, these cells secrete cytokines to augment the immune reaction, regarding the ability of TPO to cross the placenta, it can do so and cross the placenta but its effect on the conceptus is not clear.[17]

Neonatal thyrotoxicosis is caused by TRAb when they crossed the placenta.[19]

TRABs are detected in 82.05% of all GD patients who are toxic at the recruitment, and 64.81% who are euthyroid and metabolically controlled. De carvalh and coworkers have found that TRAb is positive in 90% of GD patients and 0%–20% of Hashimoto's disease and 10%–75% of atrophic thyroiditis.[20]

The presence of blocking TRAb may cause myxoedema.[21]

High titer of TRAb predicts relapses and favor the need for thyroidectomy or radioiodine.[22]

TRABs are IgG and can be subclassified to IgG G1, 2, 3, 4 but the dominant one in GD is IgG G1 as the main player in humoral immunity which take place early in the course of the disease followed by the cellular immunity imposed by Th1 and Th2 cells, fractionation of IgG to subgroups and finding IgG1 as the dominant subclass was done by using affinity chromatography in which mouse monoclonal antibodies are utilized.[23,24]

Conclusion

Eye signs are found to be more in toxic uncontrolled GD patients versus the metabolically controlled.

Both goiter and eye signs are found by treating physician as the predictor of GD but goiter is found to be higher.

TSH-receptor antibodies are the highest among toxic GD patients but they are lower in metabolically controlled patients.

TPO antibodies are high in GD patients than in healthy counterparts.

The most sensitive and specific marker in the diagnosis of GD is TSH-receptor antibodies, while the least are TPO antibodies.

Recommendation

It is recommended to conduct a prospective well-designed study enrolling a large number of thyrotoxic patients in many centers (multicenter), to study these markers in different visits and it may be fruitful to focus on predictors of relapses in such study, at the same time paying attention to other confounding factors such as the environmental ones (for example, dietary iodine) that may have an impact one the studied variables and the frequency of relapses to plan the ideal modality of treatment.

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Conflicts of interest

There are no conflicts of interest.

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