
Thyroid Peroxidase and Thyroglobuline Antibodies as a Marker in Autoimmune Primary Hypothyroidism

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Abstract:

Background: Autoimmune thyroid diseases are characterized by antibodies (Abs) directed to thyroglobuline (TG) and thyroperoxidase (TPO).

Subject & Method: This study was conducted to assess and measure the frequency and the level of the TgAb and TPOAb in sera of 25 patients with autoimmune primary hypothyroidism compared to 15 healthy controls by application of ELISA technique.

Results: The results observed that both TgAb (56%) and TPOAb (64%) of all patients were higher than that of the healthy persons TgAb(6.7%) and TPOAb(6.7%), the highest level of both autoantibodies was recorded in 6 women patients with Hashimoto's thyroiditis (100%) with a level of TgAbs (410 IU/ml) ($P<0.0005$) and TPOAbs (525 IU/ml) ($P<0.0005$).

Keywords: Antithyroglobulin Anti-thyroid Peroxidase antibody, Autoimmune Hypothyroidism.

Introduction:

The pathogenic mechanism of autoimmune thyroiditis involves a bypass of T-cell specificity to initiate the production of autoantibodies [1]. There are three distinct autoantibodies present in human thyroiditis.

These include antithyroglobulin (Anti-Tg), antithyroid peroxidase (TPO) and antiacinar colloid antigen (CA2) antibodies [2].

Serum IgG level tends to be elevated with time and the progression of the disease [3]. Patients with thyroiditis and high IgG levels had a high titer of anti-Tg antibodies [4].

Clinical diagnosis of chronic thyroiditis is usually based on evidence of serum antibodies (Abs) to a microsomal antigen and / or TG. These two antigens are considered to be the major antigens relating to chronic thyroiditis [5].

Epidemiological studies have been suggested that both antibody titer and hypothyroidism progress vary slowly in subclinical autoimmune thyroiditis [6,7], while thyroid antibodies may appear transiently and later disappear, but the appearance of TPOAb together with elevated TSH strongly suggests progression to overt hypothyroidism [8].

Thyroid antibodies (TgAb and TPOAb) and histological evidence of thyroiditis were demonstrated in patients with thyroid lymphoma [9] and thyroid carcinoma [10] as well as patients with serological and cytological signs of thyroiditis [11].

Materials & Methods:

Twenty-five patients with hypothyroidism who were admitted to Al-Rasheed Military Hospital and many private clinics were studied. 12 patients with primary Myxoedema, 6 with Hashimoto's thyroiditis, 5 with idiopathic hypothyroidism and 2 with postablative hypothyroidism. These patients include eighteen women, age range 20-52 years with average 35.4 years and seven men, age range 22-48 years with 35.6 years average

Fifteen healthy persons (8 females & 7 males), age 20-53 years with average 37 years were included in this study as control group.

Diagnosis, historical and clinical information were recorded depend on the clinical and laboratory data including thyroid function tests (T^3 , T_4 , & TSH).

Anti-TPO and anti-Tg microplate EIA kits supplied by Sandofi Diagnostic Pasture, Inc, Cat-No-31026 for anti-TPO kit and Cat-No-31025 for anti-Tg kit, 1000 Lako Hazeltine Drive, Chaska, MN, 55318 USA.

The Enzyme Linked Immunosorbent Assay (ELISA) method applied in this study.

The mean absorbance value and the concentration of the samples can be read from the calibration curve for Anti-TPO Abs and for Anti-Tg Abs (Fig.1.2).

Statistical analysis of results performed with student-t-test, and 0.05 was considered as the level of significance.

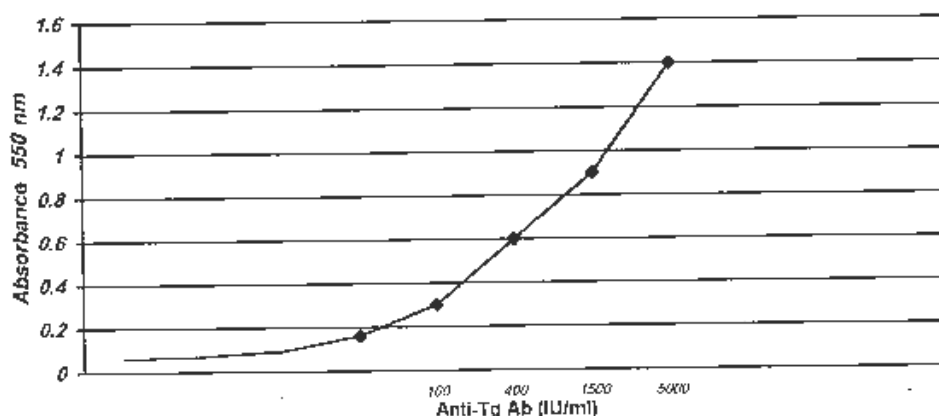


Figure 1: Calibration curve for anti Tg Ab.

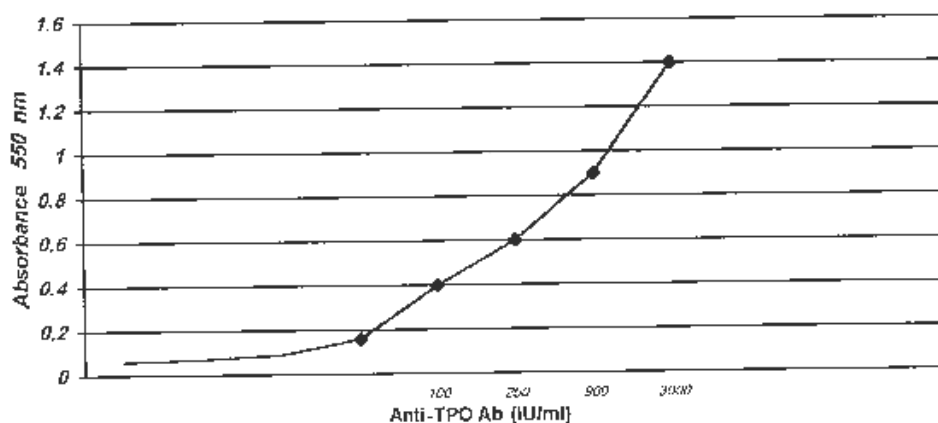


Figure 2: Calibration curve for anti TPO Ab.

Results:

The heterogenous group of patients includes primary myxoedema, Hashimoto's thyroiditis; post-ablative hypothyroidism and idiopathic hypothyroidism have been studied.

TgAbs were positive in 16 out of 25 (64%) while TPOAbs were positive in 17 (68%) of the above four types (Tab. 1& Fig.3).

Nine of twenty-five patients (36%) were positive to both TG and TPO Abs together; with 7 patients (28%) have TgAbs only and 8 patients (32%) have TPOAbs only, so there are 24 positive

results (96%) of thyroid Abs in the whole hypothyroid patients (Tab.2).

Thyroid auto-antibodies (TG and TPOAbs) were found to be positive in 10 (83.3%) and 11 (91.7%) out of 12 patients of primary myxoedema respectively. All patients with Hashimoto's thyroiditis were positive and had higher level of TG and TPOAbs. The group of idiopathic hypothyroidism included 5 patients, all have nodular goiter and all were negative for the thyroid Abs. The same applied for two cases of postablative hypothyroidism (Tab. 1& Fig 3).

Table 1: Comparison between the TG and TPO Abs frequencies using ELIAS test in total primary hypothyroidism and its subgroup, and in healthy controls.

Conditions	No	sex		Positive Tg Abs		Positive TPO Abs	
		Females	Males	No.	%	No.	%
Primary hypothyroidism	25	18	7	16	64	17	68
Primary Myxedema	12	9	3	10	83.3	11	91.7
Hashimoto's thyroiditis	6	6	-	6	100	6	100
Idiopathic hypothyroidism	5	2	3	-	-	-	-

Table 2: Comparison between the frequencies of positive results each of TG and TPO Abs using ELIS test in total Primary hypothyroidism

Conditions	No	Positive total Abs		Positive Tg Ab		Positive TPO Ab		Positive Both Abs	
		No.	%	No.	%	No.	%	No.	%
Total Primary hypothyroidism	25	24	96	7	28	8	32	9	36
Healthy controls	15	2	13.3	-	-	1	6.7	1	6.7

Table 3 and figure 3 show the mean level of Tg and TPOAbs in total primary hypothyroidism ($329 \pm 69 / 453 \pm 67$) including primary myxedema ($268 \pm 78 / 366 \pm 86$) and HT ($410 \pm 20 / 515 \pm 23$ IU/ml), while in healthy control ($37 \pm 10 / 21 \pm 8.5$ IU/ml). There is significant elevation in Tg and TPOAbs of total primary hypothyroid patients, primary myxedema patients and for Hashimoto's thyroiditis ($p < 0.0005$).

TgAbs were found to be positive in 14 out of 18 (77.7%) primary hypothyroid females with a mean level of 338 ± 68 IU/ml and they were positive in 2 out of 7 (28.5%) primary hypothyroid males with a level of 308 ± 74.2 IU/ml, while TPOAb found to be positive in 15 out of 18 (83.3%) primary hypothyroid females with a mean level of 456 ± 47 IU/ml and they were positive in 2 out of 7 (28.5%) primary hypothyroid males with a level of 419 ± 26.8 IU/ml as shown in table 4 figure 4.

Table 3: The mean serum Tg and TPO Ab levels (IU/ml) of primary hypothyroidism diseases comparing to healthy controls.

Conditions	No	Tg Ab (M± SD)	TPO Ab (M± SD)	P value
Primary hypothyroidism	25	329± 69	453±67	0.0005
Primary Myxedema	12	268±78	366±86	0.0005
Hashimoto's thyroiditis	6	410±20	515±23	0.0005
Healthy controls	15	37±10	21±8.5	-

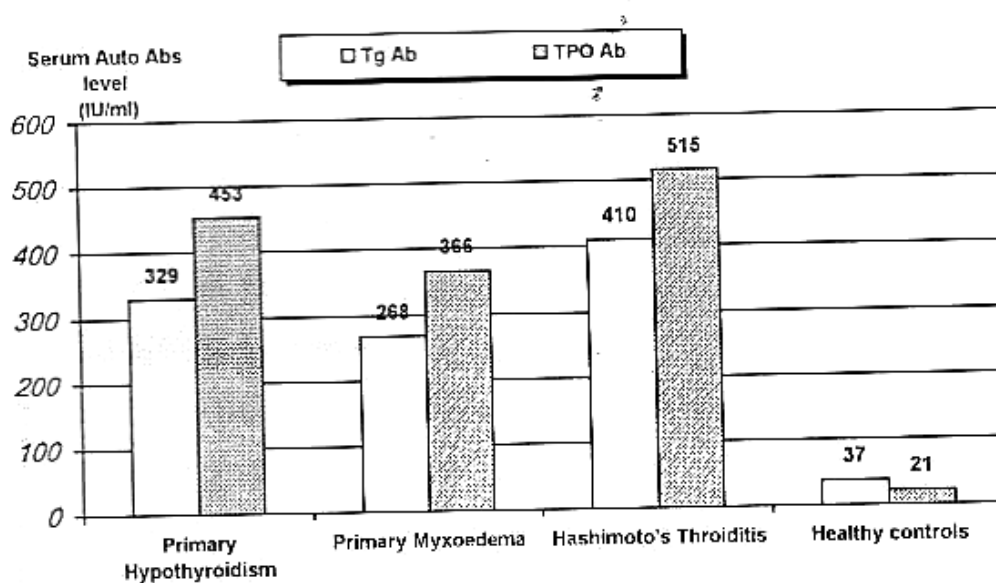
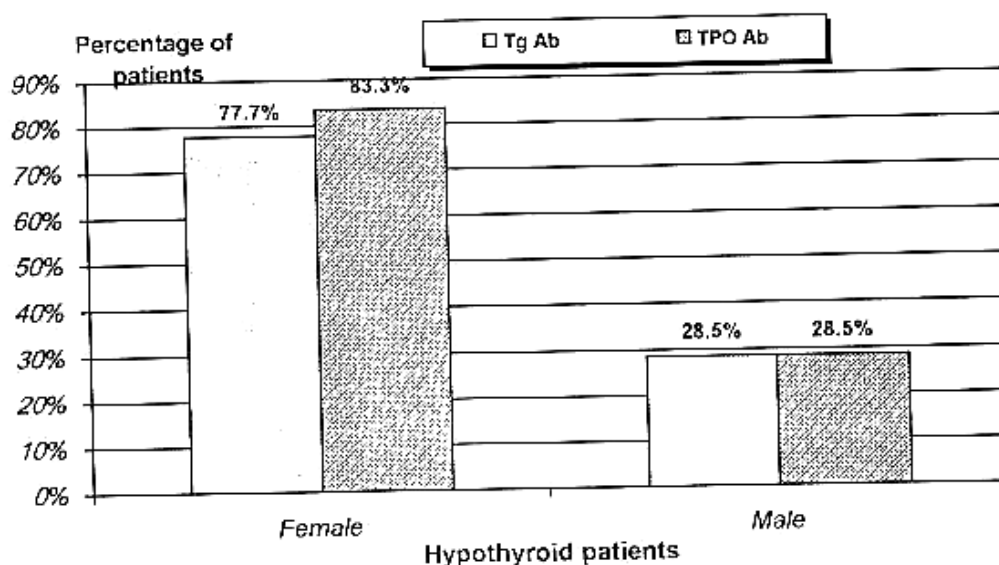
**Figure 3: Serum Tg Ab and TPO Ab levels (IU/ml) of hypothyroidism patients compared to healthy controls.**

Table 4: Comparison between the frequencies of positive results of each of Tg and TPO Abs the mean level (IU/ml) in female and male sexes of primary hypothyroidism disease and healthy controls.

Conditions	female				Males			
	Tg Ab		TPO Ab		Tg Ab		TPO Ab	
	No (%)	(M±SD)	No (%)	(M±SD)	No (%)	(M±SD)	No (%)	(M±SD)
Total Primary hypothyroidism	14/18(77.7%)	338±68	15/18(83.3%)	456±37	2/7(28.5%)	308±74.2	2/7(28.5%)	419±26.8
Healthy controls	1/15(6.75%)	37±10	1/15(6.7%)	21±8.5	0/15(0%)	37±10	1/15(6.7%)	21±8.5

**Figure 4: Percentage of Tg Ab and TPO Ab in different sexes of hypothyroid patients.****Discussion:**

The presence in serum of TPO and Tg antibodies in serum is almost invariably associated autoimmune thyroid diseases [7].

Many authors investigated the frequency of antithyroid antibodies in chronic thyroiditis using different assay methods, and demonstrated a significant increased TPO and Tg antibodies in

sera from patients with Hashimoto's thyroiditis (HT) [4,6,7,8,12,13]. Weetman (1990) [4], and Mitsuma (1999) [13] demonstrated the increase frequency and mean level of autoantibodies in hypothyroidism patients. These findings are in accordance with our results of increased TPO and Tg antibodies sera from patients with primary hypothyroidism. In addition, our results were in

consistent with that of Nakamura & Binder (1988) who reported that the highest level of thyroid antibodies appears in autoimmune thyroiditis especially HT and the lowest level appear in healthy controls ^[14].

In contrast, Aho *et.al* (1985)[7] found a lower prevalence of TPOAbs (73%) and Tg Abs (40%) in patients with HT compared to our results which was 100% for each TPO and Tg Abs. Their results were in contradictory to ours, but differences in the assay procedure might explain these discrepancies ^[15].

The frequency of positive results for thyroid and antibodies in healthy subjects may fluctuate from one study to another. Shimojo *et.al* . (1987) ^[16] and Naito *et.al* (1990) ^[17] by using ELISA method, reported a frequency of positive TPO and Tg Abs in sera from healthy subjects of 14.3% and 12.7% respectively. In contrast, Scherbaun *et.al*.(1987) ^[9] by using haemagglutination (HA) method, reported a frequency of positive results from normal controls in 3.8% and 1.3% for TPO and Tg Abs respectively. In the present study, the positive results for both Abs in normal controls were lower than that reported by Shimjo *et.al*. and Naito *et.al* And higher than that reported by Scherbun *et.al* ^[9,16,17].

The possible explanation is that Shimojo *et.al*. and Naito *et.al*. in their studies did not exclude healthy subjects with a positive family history for autoimmune disease ^[18]. On other hand, the prevalence of thyroid Abs depends on detection methods ^[15]. This can explain the higher sensitivity of ELISA method that we used in our study.

Furthermore, the TPO Abs occurs frequently in human autoimmune thyroid disease and observations, in vivo and in vitro, that their presence is closely associated with thyroid destruction and development of hypothyroidism ^[2]. Our results agree with Cayzer *et.al*. ^[12] and Yasso ^[8].

Finally, the sex variation is known to be important aetiology and incidence of many diseases, including autoimmune thyroiditis ^[14]. Dingle *et.al* ^[19] as well as Doniach & Roitt ^[20] reported the incidence of positive Abs in females is about three time that in males. Our results regarding the frequency and levels of thyroid Abs in different sexes were consistent with the previous reports.

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