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Autoimmune Hashimoto Thyroiditis with Special Reference to Anti-Thyroid Peroxidase Antibodies

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Abstract:

Background: *Chronic autoimmune (Hashimoto's) thyroiditis is the most common and extensively studied organ-specific autoimmune disorder in humans. It is the most common cause of hypothyroidism in iodine-replete areas of the world. However, in clinical practice, the diagnosis of the disease is based on positive serum thyroid autoantibodies and the characteristic echo graphic pattern of diffuse or irregular hypoechogenicity of the thyroid gland.*

***Aim*:** *The aim of the present study was to define the prevalence of (TPO) antibodies and autoimmune thyroiditis in people with a normalized iodine intake and detection of Hashimoto disease by using (TPO).*

Material and *Method*: *100 patients who had symptoms of thyroid gland malfunction, at King Khalid Hospital and Maternity hospital in Hail city, Saudi Arabia, from October 2013 to February 2014. Three mL of venous blood were collected for detection of Anti-thyroid peroxidase (anti-TPO) antibodies in patient serum by using TPO.*

Result: *The obtained result revealed that, about 11 participants were suffered from Hashimoto thyroiditis in Hail region with average age of (7 to 40 years old), 20% of them were male and 80% female and 37% were positive to Electrochemiluminescence anti-TPO assay with titer ranged from (5 to 600 IU/ml).*

***Conclusion*:** *From this Study we concluded that have 11% people live in Hail suffered from Hashimoto thyroiditis.*

Keywords: *Autoimmune, (AIT), Thyroid, iodine deficiency, ELISA, TPO, Hashimoto disease.*

1. INTRODUCTION

Autoimmune thyroid disease is the commonest category of autoimmune disease in humans. The two leading types are Hashimoto's type autoimmune thyroiditis (AIT), including the atrophic form, which presents as primary myxedema, and autoimmune thyroiditis, which is also known as Graves' disease, The commonest form of autoimmune thyroiditis (AIT) is hypertrophic thyroiditis of the Hashimoto type **[1].** Hashimoto thyroiditis is characterized by the loss of thyroid cells and gradual destruction of the gland, leading to thyroid hormone deficiency [2]. To detect the clinical symptoms of AITD,

thyroid volume is usually determined with ultrasonography, and serum concentrations of three hormones [thyrotrophin (TSH), thyroxine (T4) and triiodothyronine (T3)] and auto antibodies against thyroidperoxidase (TPOab) and thyroglobulin (TGab) are measured **[2].** Autoimmunity against the thyroid gland results in two opposite pathogenic paths: hyperthyroidismin Graves' disease and thyroid destruction in Hashimoto thyroiditis. Increased circulating activated T cells and thyroid specific auto antibodies occur in Graves' disease. Antibodies directed against the TSH receptor stimulate thyroid function and lead to glandular over activity **[3].** Subclinical Hashimoto thyroiditis is determined by a reduced serum TSH concentration (less than 0·3m IU/ml) but with concentrations of free T3 and T4 hormones in the normal range. Under clinically presenting (over) hypothyroidism, both TSH and thyroid hormone concentrations are decreased **[4].**

Thyroid-associated ophthalmopathy (TAO) is clinically relevant in approximately 50% of patients with Graves' disease as Graves's ophthalmopathy, but it may be found in patients with no past or present history of hyperthyroidism, or even in patients who are hypothyroid because of Hashimoto thyroiditis **[5].** In contrast to some regions in Germany iodine supply has normalized in Berlin as demonstrated in newborns **[6]** and schoolchildren, which has resulted in a decrease in thyroid volumes and a very low frequency of goiter **[7].** Therefore, it can be assumed that with normalized iodine intake causes other than iodine deficiency have to be considered in children and adolescents with thyroid enlargement. The most frequent cause of juvenile goiter in populations with a normal iodine intake is proliferate autoimmune thyroiditis whish is called Hashimoto's thyroiditis **[8].**

The etiology of autoimmune thyroiditis (AIT) remains unclear. Predisposing genetic factors **[9]** seem to be evident, but environmental factors **[10]** such a stress **[11,12],** infections, trauma, smoking **[13],** drugs and nutrition, especially an increased iodine supply as well as an increased age have also been shown to be conclusively linked to AIT. A four to seven times increased prevalence is observed in females **[14],** strongly indicating hormonal influences **[15, 16].** In addition, iodine plays a key role in the manifestation of AIT **[17],** as substantiated by experimental **[15–18].**

The autoimmune process is believed to begin with the activation of CD4 (helper) T lymphocytes specific for thyroid antigens **[19].** Such antigen-specific T cells have been isolated from thyroid tissue from patients with Graves' disease **[20].**

Interferon gamma, a cytokine product of activated T cells, can induce the expression of MHC class II molecules by thyroid cells. Hence, interferon gamma released from activated T cells may induce the expression of MHC class II molecules by thyroid cells, thereby leading to T-cell restimulation by thyroid cells and perpetuation of the autoimmune process **[20, 21].** The mechanism underlying the initial activation of T cells is not clear but may be less antigen-specific than in molecular mimicry, since the thyroid itself promotes further expansion of the relevant population of T cells. Once activated, self-reactive CD4 T cells can stimulate auto reactive B cells to be recruited into the thyroid and to secrete thyroid antibodies. The three main target antigens for thyroid antibodies are thymoglobulin **[22]**, the storage protein for thyroid hormones; the thyroid microsomal antigen, which has been identified as thyroid peroxidase, the rate-limiting enzyme in thyroid hormone biosynthesis; and the thyrotrophin receptor **[23].**

Activated CD4 T cells recruit cytotoxic (CD8) T cells as well as B cells into the thyroid **[24].** The direct killing of thyroid cells by CD8 cells is believed to be the main mechanism responsible for hypothyroidism. However, thyroid autoantibodies may also have a pathogenic role **[25].**

Anti–thyroid peroxidase antibodies inhibit the activity of thyroid peroxidase in vitro but are unlikely to have a primary effect in vivo **[26].**

Anti–thyrotrophin-receptor antibodies may also contribute to hypothyroidism by blocking the action of thyrotrophin. These antibodies have been reported in about 10 percent of patients with goitrous autoimmune thyroiditis and in about 20 percent of those with atrophic autoimmune thyroiditis **[27, 28].**

So far, no studies have been presented that have assessed the influence of the increased iodine intake on the prevalence of anti-thyroid peroxidase (TPO) antibodies and autoimmune thyroiditis in a prospective study in the German population. Since scanty data are available in the literature on the prevalence of anti-TPO antibodies in children and adolescents without an increased risk for autoimmunity, we decided to assess the iodine excretion as well as the prevalence of anti-TPO antibodies in a large cohort of healthy children and adolescents **[27, 28].**

Moreover, these data were correlated to the results of ultrasound studies. In order to include a large number of patients a method for the measurement of antibodies to thyroid peroxidase (anti-TPOAb) in blood spots was established. Hashimoto, permanent hypothyroidism is not inevitable. Progressive disease appears to be signaled by a high titer of thyroid antibodies and elevated serum thyrotrophin concentrations, especially in patients over the age of 45 years and in association with thyroid atrophy. The underlying mechanisms responsible for initiating thyroid autoimmunity and promoting the progression of the disease remain unknown **[29].**

1.1. Aim of this Study

- To determine the seroprevalence of Hashimoto autoimmune thyroiditis in Hail region, Saudi Arabia.
- To detect the level of anti-thyroid peroxidase antibodies in patient serum by using of anti-TPO immunoassay.

2. MATERIAL AND METHODS

2.1. Study Population

The study population was 100 patients who had symptoms of thyroid gland malfunction, at King Khalid Hospital and Maternity hospital in Hail city, Saudi Arabia, from October 2013 to February 2014.

Since the study populations were patients, the purpose and procedures of the study were explained written informed consent was obtained from all patients before the procedure.

2.2. Questionnaires

The questionnaires included questions on Age, gender, crowded family living, occupation and educational level, the presence of thyroid disorder, familial thyroid disorders and iodine supplementation.

Written approvals were collected and face-to-face interviews were conducted with the participating patients, during which the questionnaire form was filled out. The data was obtained through the questionnaire after receiving the necessary permission from the institution, the data collection process began. Patients were asked whether they had experienced any thyroid problems to research the association between current thyroid symptoms and autoimmunthyroditis positivity.

2.3. Anti-Thyroid Peroxidase (Anti-TPO) Antibodies in Autoimmune Thyroid Disease

2.3.1. Serum Samples

Three mL of venous blood were collected from 29 participants, 23 female and 6 male, serum was used for detection of autoimmune thyroid disease.

2.3.2. Electro chemiluminescence anti-TPO assay (COBAS Elecsys®)

The Elecsys Anti-TPO immunoassay was used for the invitro quantitative determination of antibodies to thyroid peroxidase in human serum. This is an immunoassay with streptavidin microparticles and electro chemiluminescence detection. First and second incubations were nine minutes in duration. There action mixture was aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode, unbound substances were removed. Voltage is applied to the electrode inducing chemiluminescent emission which is measured by a photomultiplier. Results were determined by using a calibration curve that is generated specifically on each instrument by a 2 point calibration and a master curve provided with their agent bar code.

2.4. Statistical Analysis

Statistical analysis was performed by using SPSS for Windows version 12.0 (SPSS INC., Chicago, III., USA). Data was presented as mean ± SD. Chi-square analysis μ^2 was used in findings on comparison of autoimmune thyroditis positivity according to individual characteristics. Evaluation was carried out at the 95-99% confidence interval and $P< 0.05$ was considered statistically significant.

3. RESULT

3.1. Prevalence of Hashimoto Thyroiditis in Hail Region

Prevalence o Hashimoto thyroiditis was detected in Hail region, during the period between November, 2013 and April, 2014. The highest number of infected patients was detected in March 2014 was 19 patients as shown in **Figure (1)**.

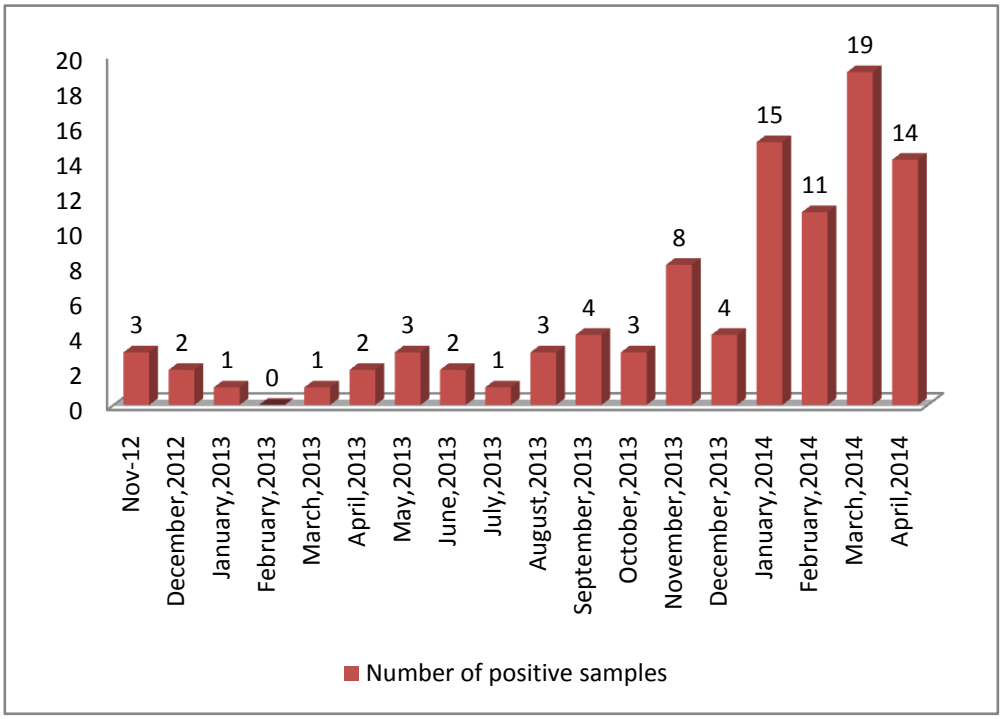

Fig1. *Prevalence of Hashimoto thyroiditis in Hail region.*

3.2. Comparison of Demographic Characteristics and Hashimoto Thyroiditis Positivity

During October 2013 to February 2014, 100 participants were enrolled in this study, 22 male and 78 female. About 11 participants were suffered from Hashimoto thyroiditis, it was found that, the average age of the patient was (7 to 40 years old), 20% of them were male and 80% female, 32% of them unmarried and 68% married, as shown in **Table (1)**.

Table1. *Comparison of demographic characteristics and Hashimoto thyroiditis positivity.*

Demographic and socioeconomic characteristics		**Hashimoto thyroiditis + (n=11) N0.(%)[1)]**	**Hashimoto thyroiditis – (n=89) N0.(%)[2)]**	χ^2	**P***
Age	7-10 11-20 21-30 31-40	2(18.1%) 1(9.0%) 0(0%) 8(27.7%)	1 (1.12%) 11 (12.35%) 27(30.33%) 50(56.17%)	7.162	0.0669
Gender	Male Female	1(9.0%) 10(90.90%)	19(21.34%) 70(78.65%)	0.919	0.338
Family situation	Unmarried married	3(27.27%) 8(72.72%)	29(32.58%) 60(67.41%)	0.127	0.722
Occupation	Job No job	3(27.27%) 8(72.72%)	31(34.83%) 58(65.16%)	0.249	0.618
Educational level	Primary high school university	6(54.54%) 2(18.18%) 3(27.27%)	18(20.22%) 16(17.97%) 55(61.79%)	6.817	0.033

[1)] *Percentage in Hashimoto thyroiditis (+)s.*

[2)] *Percentage in Hashimoto thyroiditis (-) s.*

**Percentage statistically significant difference (P>0.05).*

3.3. Comparison of Knowledgement about Hashimoto Thyroiditis and Hashimoto Thyroiditis Positivity

The obtained data revealed that, two infected patients know about Hashimoto thyroiditis while one of them know about the test for detection of Hashimoto thyroiditis and know about importance of presence iodine in his food as shown in **Table (2)**.

Table2. *Comparison of Knowledgement about Hashimoto thyroiditis and Hashimoto thyroiditis positivity.*

Knowledgement about Hashimoto thyroiditis[1)]	**Hashimoto thyroiditis + (n=11) no. (%)[2)]**	**Hashimoto thyroiditis – (n= 89) no. (%)[3)]**	**χ^2**	**P***
I know about Hashimoto thyroiditis infection	2(18.18%)	10(1.12%)	0.447	0.504
I Know about the test for detection of Hashimoto thyroiditis	1(9.0%)	7(7.86%)	0.02	0.888
I know about importance of presence iodine in my food.	1(9.0%)	17(19.10%)	0.665	0.415

[1)] Ones who answered yes in Knowledgement about Hashimoto thyroiditis were considered.

[2)] Percentage in Hashimoto thyroiditis (+) s.

[3)] Percentage in Hashimoto thyroiditis (-) s.

**Percentage statistically significant difference (P>0.05).*

3.4. Comparison of Medical History And Hashimoto Thyroiditis Positivity

Obtained results revealed that, 11 patients infected with Hashimoto thyroiditis and all of them conduct hormonal test for detection of thyroid hormone and Anti-TPO test for detection of anti-thyroperoxidase antibodies as shown in **Table (3)**.

Table3. *Comparison of medical history and Hashimoto thyroiditis positivity.*

Serological test for detection of *Hashimoto thyroiditis*[1)]	**Hashimoto thyroiditis + (no=11) no. (%)[2)]**	**Hashimoto thyroiditis – (no=89) no. (%)[3)]**	**X^2**	**P***
Infection with Hashimoto thyroiditis	11(100%)	0(0%)	100	0.00
I conduct hormonal test for detection of thyroid hormone in serum.	11(100%)	65(73.03%)	3.903	0.0482
I conduct Anti-TPO test for detection of anti-thyroperoxidase antibodies in serum	11(100%)	18(20.22%)	30.26	0.00

[1)] Ones who answered yes in medical history about Hashimoto thyroiditis were considered.

[2)] Percentage in Hashimoto thyroiditis (+)s.

[3)] Percentage in Hashimoto thyroiditis (-)s.

**Percentage statistically significant difference (P>0.05).*

3.5. Detection of Anti-Thyroid Peroxidase (Anti-TPO) Antibodies in Patient Serum

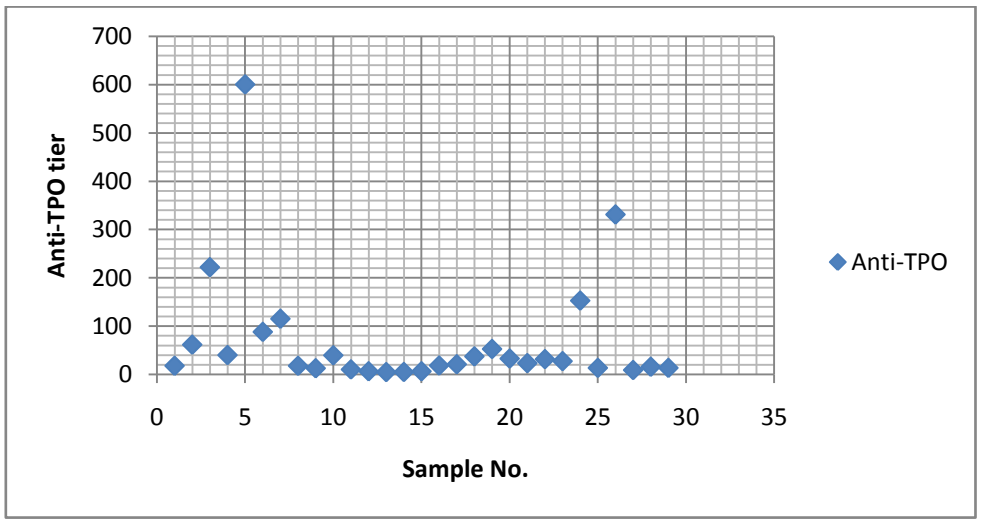

Fig2. *Titer of Anti-thyroid peroxidase (anti-TPO) antibodies in patient serum*

About 29 serum samples were test by using of Electrochemiluminescence anti-TPO assay, about 11 samples were positive for Anti-TPO assay with titer ranged from (5 to 600 IU/ml), as shown in **Table (4)** and **Figure (2)**.

Table4. *Detection of Anti-TPO antibodies in patient serum.*

Samples No.	Sex	Age	Anti-TPO (IU/ml)	Results
1	F	49	18	Normal
2	M	52	61.78	High
3	M	33	222	High
4	F	11	40.25	High
5	F	38	600	High
6	F	7	88.18	High
7	F	9	115.3	High
8	F	30	17.99	Normal
9	F	31	12.91	Normal
10	F	34	39.7	High
11	M	18	10.5	Normal
12	F	41	6.67	Normal
13	F	11	5	Normal
14	F	15	5.2	Normal
15	F	35	6.67	Normal
16	F	27	18.56	Normal
17	M	44	21.2	Normal
18	F	55	37.43	High
19	F	60	52.8	High
20	F	39	33.00	Normal
21	F	52	23.5	Normal
22	M	30	31.78	Normal
23	F	20	27.6	Normal
24	F	45	152.81	High
25	F	38	13.7	Normal
26	F	42	331.00	High
27	F	46	9.26	Normal
28	M	49	16.00	Normal
29	F	20	13.71	Normal

4. DISCUSSION

The goals of this pilot study were to assess the seroprevalence of Hashimoto thyroiditis in Hail, Saudi Arabia and to get estimated the titer of Anti-thyroid peroxidase (anti-TPO) antibodies in patient serum. However, the obtained results from this study show that about 11% of patients who included in this study were suffered from Hashimoto thyroiditis**.** These finding was agree with a retrospective analysis of 4185 consecutive fine needle aspirates (FNA) of the thyroid over an eight year period at a teaching hospital in Kuwait revealed that 14.3% of all the aspirates had features of chronic lymphocytic (Hashimoto's) thyroiditis (HT) In Kuwait **[30].**

In Japanese women, the incidence of autoimmune thyroiditis reaches a peak in the fourth decade **[31].**

This study revealed that 37% in a total population were positive for Anti-TPO assay with titer ranged from (5 to 600 IU/ml). a frequency of 8% in a total population of 59 school children, measured anti-TPO-Ab with a sensitive immunological method **[32].** While another study from India, which described the prevalence of 7.2% in a large group of 1810 girls who were selected, only measured microsomal antibodies **[33].**

A study was taken place in Sardinia with moderate iodine deficiency measured also microsomal or anti- TPO-Ab with a prevalence rate of 2.92%, ranging from 0.0 to 7.3% depending on the community **[33].**

5. CONCLUSIONS

From this Study we concluded that have 11% people live in Hail region, Kingdom of Saudi Arabia were infected with Hashimoto thyroiditis, 20% of them were male and 80% female. About 37% of total populations were positive for Anti-TPO assay with titer ranged from (5 to 600 IU/ml).

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