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Study of thyroid immunological and functional disorders in women with unexplained infertility

Ali A. Bendary[1] and Mohamed E. El Hodiby[2]
[1]Department of Obstetrics & Gynecology, Faculty of Medicine, Benha University, Benha, Egypt
[2]Department of Obstetrics & Gynecology, Faculty of Medicine, M.U.S.T. University, Giza, Egypt

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SUMMARY

Aim: Autoimmune thyroid disease (AITD) is characterized by the presence of antithyroid antibodies: these antibodies have been reported to exist in 5-10% of reproductive-aged women. Thyroid dysfunction can cause infertility. We here attempted to determine possible relationship between thyroid disorders/autoimmunity and unexplained infertility.

Methods and Results: We retrieved data on women who visited us and analyzed them. Thyroid dysfunction was observed in 32.9% and 3.4% for the infertile and control groups, respectively. In women with unexplained infertility 67.1% were euthyroid, 11.4% were subclinical hypothyroidism, 12.9% were hypothyroidism and 8.6% were hyperthyroidism, while in fertile control group 96.6% were euthyroid and 3.4% were subclinical hypothyroidism. Thyroid antibodies were present in 18.6% and 0% for the infertile and control groups, respectively. There was positive correlation between the presence of thyroid antibodies (TPO-ab and TG-ab) and mean duration of infertility.

Conclusion: Thyroid dysfunction, whether subclinical or overt, might be a risk factor for unexplained infertility. The presence of thyroid autoantibodies in euthyroid asymptomatic women may serve as a marker for unexplained infertility. Screening for thyroid dysfunction and thyroid autoimmunity may be recommended for unexplained infertility

Keywords: Autoimmune thyroid disease; Infertility; Thyrotropin

Address for correspondence:

Mohamed E. El Hodiby

Lecturer at Department of Obstetrics & Gynecology - Faculty of Medicine, M.U.S.T. University, Egypt

Orcid number
https://orcid.org/0000-0003-0968-1204
Email: ahramat@yahoo.com

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INTRODUCTION

Thyroid hormones interact with both estrogens and progesterone to maintain a normally functioning uterus and are necessary for the normal maturation of the oocytes. The impact of thyroid hormones has been reported to be both direct through the presence of thyroid hormone receptors on the ovaries and indirect through an impact on the secretion of sex hormone-binding globulin (SHBG), prolactin and luteinizing hormone-releasing hormone (LH-RH) [1]. Thus, both a normal thyroid function and immune system are thus necessary to obtain normal fertility [2].

Female causes of infertility account for 35% of all couples, male related factors for 30%, a combination of both for 20% and unexplained infertility for 15% [3,4].

The principal causes of infertility in females are endometriosis, tubal occlusion, and ovulatory dysfunction (OD) [5].

Infertility associated with ovarian dysfunction relates to a heterogeneous group of disorders (WHO I hypogonadotropic; WHO II normogonadotrophic; WHO III hypergonadotrophic) [6].

The major factors that establish uterine receptivity for implantation and further embryo development are progesterone, estrogens and the immunological system, thus thyroid hormone abnormality may have a role [7].

The diagnosis of unexplained infertility is reached after doing all investigations in order to exclude any organic cause of infertility including documentation of adequate ovulation, normal hormonal profile, tubal patency, and normal uterine cavity by ultrasound hysterosalpingogram or laparoscopy and normal semen analysis [8].

Infertility and reproductive impairment can be compromised by abnormalities in both the endocrine and the immune system. Women with thyroid dysfunction often have menstrual irregularities, infertility, and increased morbidity during pregnancy [9].

Thus, thyroid function, especially autoantibody, is suspected to be strongly associated with unexplained infertility, but data is not sufficient. So, we here attempted to determine this through this study.

Aim of the work

This study was designed to study possible relationship

between thyroid disorders, as well as thyroid autoimmunity, and unexplained infertility.

SUBJECTS & METHODS

This study was prospective study carried out in the infertility Clinics, University Hospital from October 2020 to February 2022.

It included 100 females were classified as follows:

Group I

Infertile group: 70 infertile females who were recruited from infertility clinic. They were diagnosed as unexplained infertility, age range 20-45 years old.

Group II

Control group: 30 healthy fertile females without any goiter and without any clinical symptoms and signs of thyroid dysfunction, age range 20-45years old.

Methods

All patients and control subjects were submitted to the following:

1. Full history taking
2. Full clinical examination
3. Investigations:

A. Panel investigations to infertile females in infertility clinic:

- Hormonal investigations for FSH, LH, prolactin, estrogens and progesterone
- Histosalpingo graph (H.S.G.)
- Hysteroscopy
- Laparoscopy
- Ultrasonography (U/S) either abdominal or vaginal for uterus, ovaries and tubes
- Semen analysis for husband for count, motility and abnormal forms
- Previous Assistant Reproductive Technology (ART)

B. Specific laboratory investigations:

- Thyroid-stimulating hormone (TSH)
- Free thyroxin (FT4)
- Free Triiodothyronine (FT3)
- Anti-thyroperoxidase antibodies (TPO-ab)
- Anti-thymoglobulin antibodies (TG-ab)

Sampling

1. The specimens should be blood serum type and usual precaution in collection of venipuncture samples should be observed.
2. Allow blood to clot.
3. Centrifuge the specimen to separate the serum from cells and stored in refrigerated at 2-8˚C for 5 days and at -20˚C for up to 30 days.
4. Investigation of sample with each reagent for (TSH, FT4. FT3. TPo-ab, TG-ab) by ELISA.

Measurement of free triiodothyronine (FT3)

The Quantitative Determination of Free Triodothyronine Concentration in Human Serum by a Microplate Enzyme immunoassay. Normal references range of FT3: (3.5 - 9 Pg/mL) [1].

Principle: Competitive enzyme immunoassay-Analog method for free T3: The essential reagents required for a solid phase enzyme immunoassay include immobilized T3 antibody, upon mixing immobilized antibody, enzyme-T3 conjugate and a serum containing the native free T3 antigen, a competition reaction results between the native free T3 and the enzyme-T3 conjugate for a limited number of insolubulized binding sites.

Measurement of thyrotropin (TSH)

The Quantitative Determination of Thyrotropin Concentration in Human Serum by a Micro plate Immunoenzymo metric assay. Kit (monobind Inc. lake Forest, CA 92630, USA). Product code: 325-300.

Normal references range of TSH: (0.27-4.2 mIu/L).

The cut of value of TSH is 2.5 ng/dL (1).

Principle: Immunoenzymoinetric assay: The essential reagents required for an immunoenzymomelric assay. Include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and: distinct epitope recognition, in excess and native antigen, and exogenously added biotinylated monoclonal anti-TSH antibody., reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

Measurement of free thyroxine (fT4)

The quantitative determination of free thyroxine concentration in human serum by a micro plate enzyme immunoassay.

Normal references range of FT4: (9.3 – 30 Pg/dL).

Principle: Competitive enzyme immunoassay-Analog method for free T4: Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and the enzyme-antigen conjugate for a limited number of Insolubulized binding sites.

Measurement of anti-thyroid peroxidase (Anti-TPO):

The quantitative determination of thyroid peroxidase

(TPO) auto antibodies in human serum or plasma by a micro plate enzyme immunoassay.

Normal references range of TPO-ab: (0 – 40 Iu/L).

Principle: A sequential ELISA method: The reagents required for the sequential EUSA assay Include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. Upon mixing biotinylated antigen and a serum containing the autoantibody, reaction results between the antigen and the antibody to form an immune-complex.

Measurement of anti-thyroglobulin (Anti- TG):

The quantitative determination of thyroglobulin (tg) auto antibodies in human serum or plasma by a micro plate enzyme immunoassay.

Normal references range of TG-ab: (0 – 125 ug/L)

Principle: A sequential ELISA method (TYPE 1): The reagents required for the sequential ELISA assay include Immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a micro plate well through the interaction of streptavidin coated on the well and exogenous!/added biotinyiated thyroglobulin antigen.

Test procedure for all tests:

Before proceeding with the assay, bring aft reagents, serum references and controls to room temperature (20-27 V).

1. Format the micro plate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused micro well strips back into the aluminum bag, seal and store at 2-8˚C
2. Pipette 0.050 ml (50 ul) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100 ul) of (fT3, TSH, FT4, anti-TPO and anti TG) Enzyme Conjugate solution to all wells. According type of test.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300 ul of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's Instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100 ul) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells

Do not shake plate after substrate addition

1. Incubate at room temperature for fifteen (15) minutes.
2. Add 0.050ml (50 ul) of stop solution to each well and gently mix for 15-20 seconds.). Always add reagents in the same order to minimize reaction time differences between wells
3. Read the absorbance in each well at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

RESULTS

Tab. 1. showed that, the frequency of different thyroid disorders in women with unexplained infertility was overt hypothyroidism 9 (12.9%), subclinical hypothyroidism 8 (11.4%), overt hyperthyroidism 6 (8.6%), euthyroid with +ve thyroid auto antibodies 10 (14.28%) and subclinical hypothyroidism with +ve thyroid autoantibodies 3 (4.28%), compared to control group which had subclinical hypothyroidism 1 (3.4%). So the overall frequency of thyroid dysfunction was higher in infertile group compared to control group [32.9% and 3.4% respectively P=0.005].

Tab. 2. showed that, the frequency of positive thyroid autoantibodies was higher in the infertile group compared to control group [13 (18.6%) and 0 (0%) respectively P=0.009]. According to median range of thyroid autoantibodies among study groups, TPO-ab was significantly higher in infertile group when compared to fertile group, 23 Iu/L and 21 Iu/L respectively (P=0.02), but there was no significant difference as regard TG-ab in infertile and fertile groups (P >0.05).

Tab. 3. showed that, subclinical hypothyroidism with +ve thyroid autoantibodies had higher mean duration of infertility when compared with euthyroid, subclinical hypothyroidism, hypothyroidism, hyperthyroidism, euthyroid with +ve thyroid autoantibodies groups [(7.66 years VS 3.4, 5.8, 2.8, 3.6, 4.35 years) respectively P=0.004]. There was significant difference between subclinical hypothyroidism with +ve thyroid autoantibodies group and euthyroid, hypothyroidism, hyperthyroidism and euthyroid with +ve thyroid autoantibodies groups [p=0.001, p=0.001, 0.006 and p=0.016 respectively], however, there was no significant difference as regard mean duration of infertility between subclinical hypothyroidism with +ve or –ve thyroid autoantibodies. (P >0.05).

Tab. 4. showed that, hypothyroidism has significantly higher TSH level when compared with others groups [(6.1 mIu/L VS 1.2, 2.9, .16, 1.27, 2.6 mIu/L) respectively

Tab. 1. Frequency of thyroid profiles among study groups.

Thyroid Profiles	Infertile		Fertile		X2	P
Euthyroid	37	52.8%	29	96.6%	0.032	0.005*
Sub clinical hypothyroidism	5	7.14%	1	3.4%	-	-
Hypothyroidism	9	12.9%	0	0%	-	-
Hyperthyroidism	6	8.6%	0	0%	-	-
Euthyroid with +ve Thyroid auto antibodies	10	14.28%	0	0%	-	-
Subclinical hypothyroidism with +ve Thyroid auto antibodies	3	4.28%	0	0%	-	-
* P is considered significant at 0.05.						

Tab. 2. Frequency of thyroid autoimmunity and Median range of thyroid autoantibodies (thyroid auto antibodies) among study groups.

Frequency of Thyroid Autoimmunity	Infertile		Fertile		X^2	P
-veThyroid auto antibodies	57	81.9%	30	100%	F	0.009
+veThyroid auto antibodies	13	18.6%	0	0%		
Level of Thyroid Autoantibodies	**Infertile Median (Range)**		**Fertile Median (Range)**		**Mann Whitney U**	**P**
TPO-AB	23 Iu/L (2-75)		21 Iu/L (3-33)		2.327	0.020
TG-ab	56.5 Iu/L (10-183)		53 Iu/L (2.7-72)		1.422	0.155
* P is considered significant at 0.05.						

Tab. 3. Mean duration of infertility in different thyroid disorders among infertile groups.

Thyroid Disorders	No.	Mean ± SD	F	P
Euthyroid	37	3.4 ± 1.9 years	3.82	0.004
Subclinical hypothyroidism	5	5.8 ± 3.0 years		
Hypothyroidism	9	2.8 ± 1.19 years		
Hyperthyroidism	6	3.6 ± 3.3 years		
Euthyroid with +ve Thyroid autoantibodies	10	4.35 ± 1.7 years		
Subclinical hypothyroidism with +ve Thyroid autoantibodies	3	7.66 ± 1.15 years		
Total	70	3.88 ± 2.22 years		
* P is considered significant at 0.05.				

P=0.000], and give highly significant difference with euthyroid, subclinical hypothyroidism, hyperthyroidism, euthyroid with +ve thyroid autoantibodies and subclinical hypothyroidism with +ve thyroid autoantibodies groups P= 0.000.

Tab. 5. showed that, there was a positive correlation between the presence of thyroid antibodies and the mean duration of infertility in infertile group, TPO-ab P= 0.04 and TG-ab P=0.001, and there was no correlation coefficient between mean duration of infertility and TSH, FT4, FT3 P>0.05.

Tab. 6. showed that, correlation coefficient between mean TSH levels and markers of thyroid autoimmunity, TG-ab and TPO-ab, in different TSH cut of value, there was significantly +ve correlation coefficient between TSH and TG-ab in TSH ranged from 0.27 to 2.5 r=0.75, P=0.05. And there was no correlation coefficient between TSH levels and markers of thyroid autoimmunity, TG-ab and TPO-ab, in others TSH levels [TSH 2.5-3.5, TSH 3.5-5, TSH >5].

DISCUSSION

Thyroid dysfunction can lead to a variety of gynecological disorders ranging from menstrual irregularities to infertility and increased morbidity during pregnancy in Women in reproductive age [10-13].

Women with elevated serum TSH levels had a lower pregnancy rate than women with a normally stimulated serum TSH [14].

In this study, the frequency of different thyroid disorders in women with unexplained infertility was overt hypothyroidism 9 (12.9%), subclinical hypothyroidism 8 (11.4%), overt hyperthyroidism 6 (8.6%), euthyroid with +ve thyroid auto antibodies 10 (14.28%) and subclinical hypothyroidism with +ve thyroid autoantibodies 3 (4.28%), compared to control group which had subclinical hypothyroidism 1 (3.4%). so, the overall frequency of thyroid dysfunction was high in infertile group (32.9%) compared to control group (3.4%). And also, the frequency of positive thyroid autoantibodies was higher in the infertile group (18.6%) compared to control group (0%).

In our study we found 8 from 70 unexplained infertile women (11.4%) had subclinical hypothyroidism, in agreement to this result,

In contrary to our result, Studies looking at the association of subclinical hypothyroidism and infertility are poorly controlled. Considering the largest cohorts published the prevalence of subclinical hypothyroidism in infertility ranges from 1 to 4% and most cases of subclinical hypothyroidism are associated with ovarian dysfunction [13].

Raber et al., (2013) were found that women who never achieved a basal TSH <2.5 mIu/l, or a TRH-stimulated TSH <20 mIu/l were observed more frequently among patients who did not become pregnant than among those

Tab. 4. Frequency of TSH in different thyroid disorders among infertile group.

Thyroid Disorders	No.	Mean ± SD	F	P
Euthyroid	37	1.2 ± 0.6 mIu/L	28.72	0.000
Subclinical hypothyroidism	5	2.9 ± 0.25 mIu/L		
Hypothyroidism	9	6.1 ± 3 mIu/L		
Hyperthyroidism	6	0.16 ± 4.9 mIu/L		
Euthyroid with +ve Thyroid auto antibodies	10	1.27 ± 0.51 mIu/L		
Subclinical hypothyroidism with +ve Thyroid auto antibodies	3	2.6 ± 8.3 mIu/L		
Total	70	1.97 ± 2.06 mIu/L		
* P is considered significant at 0.05.				

Tab. 5. Correlation coefficient between duration of infertility and thyroid profiles tests and thyroid auto antibodies among infertile group.

Tests	r	P
TSH	0.065	0.59
FT4	0.039	0.799
FT3	-0.081	0.505
TPO-ab	0.344	0.04
TG-ab	0.379	0.001
* P is considered significant at 0.05		

Tab. 6. Correlation coefficient between TSH level and markers of autoimmunity.

Markers	TSH 0.27-2.5 N. 37		TSH 2.5-3.5 N. 8		TSH 3.5- 5 N. 6		TSH > 5 N. 3	
	r	p	r	p	r	p	r	p
TG-ab	0.75	0.05*	0.4	0.92	0.06	0.91	0.5	0.66
TPO-ab	-0.36	0.43	0.37	0.37	0.23	0.65	0.5	0.66
* P is considered significant at 0.05								

who did. Subsequent abortions occurred with increased frequency with higher basal TSH, independent of the presence of AITD [12].

In our study thyroid autoimmunity were found without thyroid dysfunction in (18.6%) in agreement to this result, Poppe et al., (2012) were found that thyroid autoimmunity can be present without thyroid dysfunction and thus undiagnosed, and also found that thyroid autoimmunity among women of infertile couples (n = 438(, presenting for the First time at the Department of Reproductive Medicine had an increased risk of AITD 2.25 compared with the control population which consisted of 100 fertile women, matched for age [2].

In agreement to our result, Cramer et al., (2013) showed that TSH is a significant predictor of fertilization failure in infertile women [13].

Arajoki et al., (2017) in the Finish study reported that two spontaneous pregnancies occurred in the group after adjustment of thyroxine substitution dose, and two after initiation of thyroxine treatment for subclinical hypothyroidism, compared with none in the no thyroxine treatment group [14].

In our study we found that in 70 unexplained infertile women without previous thyroid disorders, 24.3% had increased serum TSH (both overt and subclinical hypothyroidism), in contrary to this result, Grassi et al., (2018) were found that in 129 women from infertile couples. The etiology of infertility was related to a male factor, ovulatory dysfunction and unexplained infertility. Six patients (4.6%) had serum TSH levels >4.5 mIU/l (both overt and subclinical hypothyroidism) and five of these had AITD [15].

In our study no cases in infertile and control group had subclinical hyperthyroidism, while in cohort study the prevalence of suppressed TSH (<0.1 mIu/l) in 438 women of infertile couples was 2.3%, of whom 40% had positive thyroid antibodies; 83% of these patients had normal free hormone levels (subclinical hyperthyroidism) [2].

In this study the overall frequency of thyroid dysfunction was high in infertile group (32.9%) compared to control group (3.4%). And also, the frequency of positive thyroid autoantibodies was higher in the infertile group (18.6%) compared to control group (0%), in contrary to our result Petta et al., (2017) were evaluated thyroid autoimmunity and thyroid dysfunction in women with endometriosis and found that The overall frequency of thyroid dysfunction was 12.2% and 10.8% for the Endometriosis and control groups and the frequency of positive thyroid autoantibodies, 14.9% and 22.2%, respectively [16].

In our study no cases in control group had thyroid autoimmunity so we couldn't calculated RR (Relative Risk), while in a survey by Singh et al., (2015), female patients of couples with infertility the RR of AITD is slightly but significantly increased: RR 1.95 (CI 1.50-2.53; P <0:0001) [17].

In this study, when we examined relationship between mean duration of infertility, prevalence of thyroid disorders and positive or negative thyroid autoimmunity, subclinical hypothyroidism associated with thyroid autoimmunity had higher mean duration of infertility 7.66 years, and also when mean duration of infertility increased the incidence of AITD is increased., There was positive correlation coefficient between the presence of thyroid antibodies (TPO-ab and TG-ab) and mean duration of infertility.

In agreement to our result, Grassi et al., (2018) were found that mean duration of infertility was significantly longer for patients with thyroid abnormalities (including abnormal TSH and/or AITD) [15].

The difference in these results might be explained by the presence of different causes of infertility in these studies, while in the current study no obvious causes of infertility (unexplained infertility). And also the number of cases in our study was low compared to the number of cases in these studies.

CONCLUSION

We here showed some new findings:

1. Thyroid dysfunction, whether subclinical or overt, might be an independed risk factor for unexplained infertility.
2. The presence of thyroid auto antibodies in euthyroid asymptomatic women may serve as marker for unexplained infertility.
3. Screening for thyroid dysfunction as well as thyroid autoimmunity is highly recommended in women with unexplained infertility.

We here reconfirmed the following:

1. AITD the most common autoimmune disorder affecting women in child bearing period.
2. AITD can present without thyroid dysfunction and, thus, remains undiagnosed.
3. Women in reproductive age, thyroid dysfunction can lead to a variety of gynecological disorders ranging from menstrual irregularities to infertility.

Although, these are already reported, we believe that their reconfirmation in this population is clinically important.

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