

# Antithyroid Antibodies in Sera of Women with Spontaneous Abortion and Normal Pregnancies

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*Autoimmune thyroiditis is the most frequent autoimmune disease. We analyzed the association between the presence and value of titer of anti-thyroid antibodies (atbs) and spontaneous abortion (SA). Moreover, we analyzed the association between TSH values and the presence of anti-thyroids atbs. We tested anti-TPO and anti-TG atbs in 257 women: 85 probands, 87 pregnant women with normal pregnancy outcome, and 85 with SA. We tested TSH in 87 pregnant women with normal pregnancy outcome. No significant difference was found between the prevalence of cases with positive anti-TPO and anti-TG atbs in women with normal pregnancy outcome vs. SA patients. In women with positive atbs titer, anti-TPO and anti-TG values were higher in SA group compared with normal pregnancy outcome group. A significant threshold was reached only for anti-TG atbs. TSH values were higher in pregnant women with positive anti-TPO and anti-TG values compared with those with a negative atbs titer. In pregnant women with positive anti-thyroid atbs titers, spontaneous abortion group patients have higher anti-TG titers than women with normal pregnancy outcome. TSH values are higher in the group of patients with positive anti-TPO and anti-TG atbs titers compared with the group of patients with negative anti-TPO and anti-TG values.*

**Keywords:** Anti-TPO, anti-TG, pregnancy outcome, spontaneous abortion, TSH

Anti-thyroid antibodies (atbs) belong to a family of auto-atbs which recognize self-antigens located in the thyroid [1]. Anti-thyroid atbs are of different specificities in accordance with the recognized antigen. The most studied antithyroid atbs are anti-thyroperoxidase (TPO) atbs, anti-thyreoglobulin atbs (TG), and anti-Thyroidreceptor (TRH-Rc) atbs [2]. Under certain circumstances anti-thyroid atbs could lead to a disease named autoimmune thyroiditis, which is the most frequent autoimmune disease in humans [2,3].

Anti-thyroperoxidase (anti-TPO) atbs recognize antigens that belong to an enzyme named thyroperoxidase (TPO), thyroid peroxidase or iodid peroxidase [4,5]. The role of TPO is to oxidize iodide (I-) to iodine (I) or iodinum (I+) an important step in the synthesis of thyroid hormones [6]. The TPO synthesis is upregulated by TSH and inhibited by thioamides such as propylthiouracil and methimazole [7] Anti-TPO atbs are found in certain healthy persons [3] and are associated with Hashimoto disease [2].

Anti-thyroglobulin (anti-TG) atbs recognize antigens that belong to a protein named thyreoglobulin (TG) [8]. Thyreoglobulin is a 660 kDa dimer which represents more than half of thyroid gland proteins. It is used exclusively in the thyroid [8]. Thyreoglobulin is a homodimer in which a unit contains 2778 aminoacids [9]. Thyreoglobulin is cleaved into fragments (thyrosine) which are iodinated by thyroperoxidase to generate thyroide hormones (thyroxine and triiodothyronine) [9]. TG concentration is used to monitor the recurrence of papillary or follicular thyroid carcinoma [10]. Anti-TGs are found both in healthy people and in patients with Hashimoto disease and Graves Disease [11].

Anti-Thyroid Stimulating Hormone Receptor Atbs (TRAbs) recognize epitopes located on the TSH receptor which is involved in thyroid hormone signaling. TRAbs are present in healthy people and in patients with Grave Disease [12]. TRAbs could have a stimulating or an inhibiting effect on TSH-Receptor [12].

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It is known that autoimmune atbs producing B-lymphocytes are part of the immune repertoire of healthy people [13]. In normal conditions these clones are controlled by suppressive mechanisms [14] and this is the reason why only low titers of autoimmune atbs are present in healthy persons. Factors that disturb this balance could determine an increase of autoantibody titer and an autoimmune disease [4,15].

The most frequent consequence of the presence of anti-thyroid atbs is a thyroid dysfunction: hiper- or hipothyroidia [16]. Studies were performed to analyze the association between the presence of anti-thyroid atbs and of other diseases (breast cancer [5], thyroid cancer [17], anti-gastric cells atbs [17], prolactinomas [18], systemic sclerosis [19], reumathoid arthritits [20], lupus erythematosus [21,22], helicobacter pylori infection [23], vitiligo [24], diabetes [25]) or fertility dysfunctions, too. Some of these associations are controversial such as the one between the presence of anti-thyroid atbs and infertility [26], spontaneous abortion (SA) [27] or preterm birth [28].

In the present study we aimed to analyze the association between the presence of anti-thyroid atbs and spontaneous abortion, relying on a collection of sera collected from pregnant women with normal pregnancy outcome (NPO) and with spontaneous abortion (SA).

## **Experimental part**

### *Materials and methods*

#### Patients and sera

For this study we have established three groups of patients without known history of thyroid disease: healthy asymptomatic women (HAW) (n=85), pregnant women with a normal pregnancy outcome (NPO) (n=87) and patients with spontaneous abortion (SA) (n=85). All women were asked about their medical history and morphological features.

The lot of HAW consisted of women without known thyroid pathologies and with regular menses. The group of NPO consists of pregnant women without thyroid pathologies, and with a normal course of pregnancy with birth at term. The patients with SA were patients without known thyroid pathology that had blood collected along pregnancy before the event of miscarriage. The miscarriage was documented retrospectively by the existence of a histological result from the aborted tissue.

#### Collection of sera

Sera were collected by venipuncture. The blood was collected on vacutainer with procoagulant. After separation the serum was stored in Eppendorf tubs at -80 C.

#### Measurement of anti-thyroid peroxidase (anti-TPO) antibodies, anti-thyroglobulin (anti-TG) antibodies, and TSH values

Measurement of anti-TPO and anti-Tg titer was performed by ELISA using commercially available kits from DiaMetra (Spello, Italy): anti-TPO ELISA (DK 0116), anti-TG ELISA (DK 0115), and TSH ELISA (DKO013). Anti-TPO values greater than 20 and anti-TG values greater than 5 were considered positive. An ELISA reader SUNRISE Remote, courtesy of Tecan GmbH, Austria, was used to test the sera. The method was presented elsewhere [29-30]

#### Ethical issues

The research was approved by the Committee of the University of Medicine and Pharmacy Timisoara. Informed consent was obtained from every patient.

#### *Statistical analysis*

Data are expressed in median $\pm$  Standard error of mean (SEM). GraphPad InStat software, San Diego, California, USA and SPSS, IBM Inc. were used for statistical analysis.

## **Results and discussions**

### *Prevalence of patients with positive anti-TPO and anti-TG atbs titer*

Our results show that from 257 women (85 healthy asymptomatic women, 85 with spontaneous abortion and 87 with normal pregnancy outcome), 33 (12.8%) had a positive anti-TPO atb titer, 12 (4.7%) anti-TG atbs, 10 (3.9%) anti-TPO and anti-TG atbs, and 35 (13.6%) positive anti-TPO or anti-TG atbs titers (Table 1). From 35 women that presented anti-thyroid atbs in serum, 10 cases presented both atbs positive, while 23 presented positive anti-TPO and not anti-Tg atbs, and only 2 women showed positive anti-TG and not anti-TPO atbs.

**Table 1**

PREVALENCE OF WOMEN WITH POSITIVE ANTI-TPO AND ANTI-TG ATBS TITER IN ALL 257 TESTED CASES

	anti-TPO > 20 AU /ml	anti-TG > 4 AU /ml	anti-TPO and anti-TG	anti-TPO or anti-TG
Positive titer	33 (12.8%)	12 (4.7%)	10 (3.9%)	35 (13.6%)
Total no. patients	257	257	257	257

Prevalence of cases with positive anti-TPO and anti-TG antibodies titer in women with normal pregnancy outcome (87) versus women with spontaneous abortion (85)

The analysis of prevalence of patients with a positive anti-thyroid antibodies titer (above cut-off) according to diagnosis showed no significant association with spontaneous abortion.

**Table 2**  
PREVALENCE OF PATIENTS WITH POSITIVE (ABOVE CUT-OFF) ANTI-TPO AND ANTI-TG ANTIBODIES TITER IN NPO WOMEN VERSUS SA

	anti-TPO > 20 AU /ml	anti-TG > 4 AU /ml	anti-TPO and anti-TG	anti-TPO or anti-TG
Normal pregnancy (n=87)	12 (13.8%)	6 (6.9%)	5 (5.7%)	13 (14.9%)
Spontaneous abortion (n=85)	10 (11.7%)	2 (2.4%)	2 (2.4%)	10 (11.7%)
P value	= 0.649	= 0.277	= 0.443	= 0.655

Prevalence of cases with an anti-TPO and anti-TG atbs titer twice as high as the cut-off value in the group of women with normal pregnancy outcome (87) versus women with spontaneous abortion (85)

We analyzed the number of cases with anti-TPO and anti-Tg atbs titer values twice as high as the cut-off values (table 3). The data showed no significant association of prevalence and SA.

**Table 3**  
PREVALENCE OF CASES WITH ANTI-TPO AND ANTI-TG ATBS TITER TWICE AS HIGH AS THE CUT-OFF VALUES IN PATIENTS WITH NPO VERSUS SA

	anti-TPO	anti-TG	anti-TPO and anti-TG	anti-TPO or anti-TG
Normal Pregnancy outcome (n=87)	7 (8.0%)	1 (1.1%)	1 (1.1%)	7 (8.0%)
Spontaneous abortion (n=85)	6 (7.1%)	2 (2.3%)	2 (2.3%)	6 (7.1%)
P value	= 1.000	= 0.482	= 0.482	= 1.000

Values of anti-TPO and anti TG atbs titer in sera of patients with normal pregnancy outcome vs. spontaneous abortion

If we compare data from pregnant women with normal pregnancy outcome vs. spontaneous abortion our results show that there is no significant difference between the values of anti-TPO ( $6.0 \pm 8.4$  vs.  $5.7 \pm 7.1$ , ns) and anti-TG ( $0.6 \pm 0.2$  vs.  $0.6 \pm 0.5$ , ns) atbs titer. (Table 4)

**Table 4**  
VALUES OF ANTI-TPO AND ANTI-TG ATBS TITER IN NPO VERSUS SA

Median±SEM	Normal pregnancy outcome (n=87)	Spontaneous abortion (n=85)	P value
anti-TPO	$6.0 \pm 8.4$	$5.7 \pm 7.1$	= 0.148
anti-TG	$0.6 \pm 0.2$	$0.6 \pm 0.5$	= 0.930

Anti-TPO and anti-TG atbs titer values in sera of pregnant women with autoimmune thyroiditis: pregnant women with normal outcome vs. spontaneous abortion

Our results reveal that in pregnant women with autoimmune thyroiditis (only pregnant women with anti-thyroid atbs above cut-off value) both atbs types showed higher values in the group of pregnant women with spontaneous abortion compared to pregnant women with normal outcome; a significant threshold was reached only for anti-Tg atbs. (Table 5)

**Table 5**  
ANTI-TPO AND ANTI-TG ATBS TITER VALUES IN SERA OF PREGNANT WOMEN WITH AUTOIMMUNE THYROIDITIS: SA vs NPO

Median±SEM	Normal pregnancy outcome	Spontaneous abortion	P value
anti -TPO	82.8±47.3 (n=12)	94.6±45.6 (n=10)	= 0.69
anti-TG	5.2±1.2 (n=6)	29.0±15.3 (n=2)	= 0.07

*TSH values in the group of pregnant women with and without autoimmune thyroiditis (anti-TPO and anti-TG antibodies titer above cut-off value)*

We stratified the patients according the atbs titer in five groups: the first group includes patients with negative atbs titer, while the other four groups include patients with positive atbs titers: anti-TPO (11), anti-TG (6), anti-TPO or anti-TG (12), anti-TPO and anti-TG (5) (table 8). Our data show that patients with positive anti-TPO or/and anti-TG atbs titer had higher TSH values than patients with negative atbs titers and the difference reached a significant threshold for all these patient groups. (table 6)

**Table 6**  
TSH VALUES IN PREGNANT WOMEN WITH NORMAL OUTCOME WITH POSITIVE VALUES AND NEGATIVE VALUES OF ANTI-TPO, ANTI-TG ABS, ANTI-TPO OR ANTI-TG ABS, AND ANTI-TPO AND ANTI-TG ABS

Median±SEM	TPO atbs	TG atbs	TPO or TG atbs	TPO and TG atbs
positive values	2.30±0.40 (n=11)	2.30±0.73 (n=6)	2.10±0.38 (n=12)	2.7±0.82 (n=5)
negative values	1.3±0.09 (n=71)	1.3±0.09 (n=71)	1.3±0.09 (71)	1.3±0.09 (n=71)
P value	= 0.0069	= 0.0651	= 0.011	= 0.037

Our study is the first in Romania to analyze the presence of anti-thyroid atbs in sera of women who were not recorded with thyroid pathology. Autoimmune thyroiditis is one of the most frequent autoimmune diseases affecting a significant percent of women. Several studies report different prevalence of cases with anti-TPO and anti-Tg atbs in general apparently healthy population or in patients with certain pathologies. The presence of anti-TPO and anti-TG were proved to be associated with certain diseases: breast cancer [5], diabetes [25], sclerodermia [19], vitiligo [24], etc. The results of our study show that the prevalence of anti-TPO and anti-TG atbs is similar to that reported in other countries from our area [31].

As reported in the literature, the prevalence of cases with anti-TPO atbs is more frequent than that of cases with anti-TG atbs [32]. In our study, out of 35 women that present anti-thyroid atbs, 10 cases present both positive atbs, while 23 show positive anti-TPO but not anti-Tg atbs and only 2 women showed positive anti-TG but not anti-TPO atbs. Other studies showed that anti-TG atbs are found in 50% of cases with positive anti-TPO antibodies [22].

Furthermore, we analyzed if anti-TPO or anti-Tg atbs are associated with spontaneous abortion (SA). There are many controversies surrounding the association between the presence of anti-thyroid atbs and infertility or SA. While some studies found an association between anti-thyroid autoimmunity and SA [32-34], other studies did not [35,36].

In order to study the association mentioned above, we analyzed both the prevalence of cases with positive anti-TPO and anti-TG atbs titer and the value of the atbs titer in the group of pregnant women with a normal pregnancy outcome (NPO) and with spontaneous abortion (SA).

We found no significant difference between them regarding the prevalence of cases with positive anti-thyroid atbs even if we considered the prevalence of cases with anti-thyroid atbs values higher than cut-off or twice the cut-off.

Then we analyzed if the values of the titer of anti-TPO and anti-TG atbs are associated with spontaneous abortion. For this we compared the anti-TPO and anti-TG atbs titer values in the two groups. First, we included all the cases from the groups, then only those with a positive atbs titer. No difference was found between the values of the titer of anti-TPO and anti-TG atbs in the two groups when all patients in each group were taken into account. However, when only women with autoimmune thyroiditis (an anti-TPO and anti-TG titer above cut-off value) are included in the analysis, SA group showed higher atbs titer than NPO group. Interestingly, the difference was significant only for anti-TG atbs.

Our results suggest that spontaneous abortion is not associated with the prevalence of cases with anti-thyroid atbs, but with their titer, especially for anti-TG atbs. Our study includes only a moderate number of patients and this is the reason our results cannot be generalized.

The last part of our study deals with the relationship between the anti-thyroid (anti-TPO and anti-TG) atbs titer and the thyroid function. It is known that anti-thyroid atbs could lead to autoimmune thyroiditis, thyroid tissue damage, and hypothyroidia [37]. Also, anti-thyroid antibodies could pass across placenta and reach the fetal circulation [38]. Our results show that TSH values are higher in pregnant women with autoimmune thyroiditis (AUT) (anti-TPO and anti-TG atbs above cut-off) compared with pregnant women without AUT.

This observation is especially important in pregnant women where the need for thyroid hormone increase and a subclinical hypothyroidia may decompensate [39-42]. We recommend a close follow-up of pregnant women with anti-thyroid antibodies even if they have a euthyroid function in early pregnancy [43]. It is still a debate if screening for thyroid pathology should be done [44] in simultaneous with the other screenings [45-47].

## Conclusions

Our study presents for the first time the prevalence of women with anti-TPO and anti-TG atbs in healthy probands, pregnant women with normal outcome, and women with spontaneous abortion in our country. No association between prevalence of cases with anti-thyroid atbs and spontaneous abortion was found when we considered the prevalence of women with anti-thyroid atbs with titers over cut-off or twice the cut-off values. When only women with atbs titer above cut-off values were taken into account, both anti-TPO and anti-TG titers were higher in women with spontaneous abortion compared with women with normal outcome pregnancy. Only anti-TG atbs reached a significant threshold. TSH values were higher in women with anti-thyroid atbs above cut-off compared with those with atbs values below cut-off.

*Acknowledgements: We are grateful for the publication fee supported by the Foundation for the Promotion of Health and Prevention "HELP PREVENT", CUI 38286326, Adress Str Rindunica, no 2, Resita, Romania.*

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Manuscript received: 6.09.2019