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Comparison of Pregnancy Outcomes in Pregnant Women with Positive and Negative Anti-thyroid Peroxidase Antibody

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ABSTRACT

Background & Objective: Thyroid-related adverse pregnancy outcomes can be analyzed by Anti-thyroid peroxidase antibody (Anti-TPO Ab). However, whether women with anti-thyroid peroxidase antibody (Anti- TPO Ab) positivity have a higher risk of poor pregnancy outcomes is debatable. The aim of the present study was to compare the pregnancy outcomes in pregnant women with positive and negative TPO-Ab.

Materials & Methods: This cross-sectional study was conducted on pregnant women with positive anti-TPO Ab (n= 108) and negative anti-TPO Ab (n=340) referring to Imam Khomeini Hospital and Ahvaz clinics from January 2020 to December 2020. The pregnancy outcomes, including gestational diabetes mellitus (GDM), preeclampsia, miscarriage, preterm birth, placental abruption, IUFD, IUGR, and TSH level, were compared between the two groups by SPSS software using student t-test and Chi-square test.

Results: The mean TSH level was significantly different in women with negative TPO-Ab than those with positive TPO-Ab (Mean (SD): 2.25(1.47) vs. 4.82(9.38), P<0.0001). In addition, 81 (75.00%) women in the positive anti-TPO group and 104 (30.58%) in the negative anti-TPO group had high TSH (P<0.001). The frequency of GDM in positive anti-TPO Ab pregnant women was significantly higher than that in the negative anti-TPO Ab group (46.29% vs. 34.11%, P=0.029). The results also indicated an enhancement in the rate of miscarriage (15.74% vs. 4.11%, P<0.001) and preterm parturition (13.88% vs. 3.52%, P<0.001) in positive anti-TPO Ab women.

Conclusion: Presence of positive anti-TPO Ab was significantly associated with preterm delivery, abortion and GDM in pregnancy. Treatment with Levothyroxine can reduce the risk of adverse pregnancy outcomes in women who are positive for TPOAb.

Keywords: Pregnancy Outcome, Anti-thyroid Autoantibody, Thyroid-stimulating Hormone

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Introduction

Thyroid disorders are one of the most prevalent endocrine problems in pregnant women. Subclinical hypothyroidism happens in about 2-3% of pregnant women, and 0.3-0.5% of women have overt hypothyroidism. Thyroid autoantibodies are also found in about 5-15% of women at gestational age. Based on the results of various studies, a statistically significant relationship has been reported between thyroid disorders in pregnancy and poor pregnancy outcomes **(1-3)**. Anti-thyroid peroxidase antibody (TPO-Ab) is also directly related to increasing thyroid stimulating hormone (TSH) levels. An abnormal increase in TPO Abs seems to be a marker of early diagnosis of thyroid dysfunction **(4)**. TPO acts as one of the main enzymes in the synthesis of thyroid hormones and TPO Ab antigen. An abnormal increase in TPO Ab is usually seen at the onset of thyroid dysfunction. TPO Ab is an essential indicator for diagnosing the cause of primary hypothyroidism and is the leading indicator for diagnosing autoimmune thyroid illnesses, such as Graves' disease and Hashimoto's thyroid **(5)**.

Women with positive TPO Ab pregnancy have an enhanced risk of hypothyroidism in the course of gestation. In women with positive TPO Ab before pregnancy, the risk of subclinical hypothyroidism and hypothyroidism doubles after pregnancy **(6, 7)**.

An abnormal increase in the level of TPO Ab is also a significant risk factor for other diseases, including gestational diabetes mellitus (GDM) **(6)** and anemia **(2)** during pregnancy. The association between an abnormal increase in TPO Ab levels and pregnancy

outcomes has been reported in several studies. These outcomes include miscarriage, preterm delivery, prenatal death, polyhydramnios, and placental abruption, and increased TPO Abs levels are risk factors for all of these outcomes **(8-10)**. However, in some other studies, there was no association between fertility, pregnancy rate, live birth and abortion between TPO Ab positive and TPO Ab negative patients **(1, 11)**. Whether women with anti-thyroid peroxidase antibody (TPOAb) positivity have a higher risk of poor pregnancy outcomes is debatable. Given what was stated above, the extant research was carried out to compare the pregnancy outcomes in pregnant women with positive and negative TPO-Ab.

Methods

The present study is a cross-sectional study carried out prospectively on pregnant women referring to Imam Khomeini Hospital and Ahvaz clinics from January 2020 to December 2020.

Sample size based on reference No. 6, considering the difference in TSH level between two groups, a 95% confidence interval, and 98% power was determined to be at least 264 women. The sample size was calculated based on the fallowing formula:

$$\mathbf{n} = \frac{\left(\mathbf{z}_{1-\frac{\alpha}{2}} + \mathbf{z}_{1-\beta}\right)^2 * (\boldsymbol{\sigma}_1^2 + \boldsymbol{\sigma}_1^2)}{\mathbf{d}^2} = \mathbf{264}$$

Finally, 448 women were included in the study by convenience sampling method. Only women with the age between 18 and 35 years in the first trimester of pregnancy, and with spontaneous pregnancy (without assisted reproductive techniques) participated in the research. The women with a background of autoimmune diseases were excluded from the research. Basic characteristics of patients including age, parity, body mass index (BMI), number of pregnancies, number of deliveries, number of miscarriages, and number of live children were selected by the researcher from the patient's medical records and recorded in a checklist designed to collect information.

TSH and Anti-TPO tests were performed on all eligible subjects in the first trimester of pregnancy. TSH levels in all subjects were assessed and recorded by electrochemiluminescence (ECL) method and using existing kits. Enzyme-linked immunosorbent assay (ELISA) and immunometric methods were used to measure TPO antibodies in serum. Based on the test results, the samples were separated into two groups: Negative Anti-TPO and positive Anti-TPO (Figure 1). The common range for TSH levels was 0.1-3.9 µIU/ml in the first trimester of expectancy and 0.1-4.1 µIU/ml in the second and third trimesters of pregnancy. The normal range of TPO Ab was <35 µIU/ml and the values higher or equal to this indicate the increased level of serum anti-TPO **(8)**.

All hypothyroid patients (TSH levels lower than usual) were treated with levothyroxine. The drug was administered under the supervision of an endocrinologist.

All participants in the study were followed until 42 days after the termination of pregnancy. All patients were monitored by a physician and were regularly examined by a gynecologist during the study period, and any specific complications or problems were carefully evaluated. If patients did not refer to follow-up, they were followed up via phone and called for examination, if needed.

Pregnancy outcomes including miscarriage, preterm delivery (<34 weeks), preeclampsia, GDM, placental abruption, Intrauterine Fetal Death (IUFD), and Intrauterine Growth Restriction (IUGR) were evaluated and recorded. The presence of IUGR was reported based on fetal weight assessment using ultrasound images if the fetal weight was below the 10th percentile of gestational age. Finally, the collected data of two groups of negative anti-TPO and positive anti-TPO were compared.

Intrauterine fetal death and placental abruption were diagnosed by assessing the patient's symptoms, including bleeding and pain, and performing ultrasound and monitoring fetal symptoms. GDM was defined as onset or first recognition of abnormal glucose tolerance during pregnancy. In a 75 g oral glucose tolerance test, a diagnosis of gestational diabetes mellitus is made if one or more of the following glucose levels is elevated: Fasting: ≥ 92 mg/dl, One hour: ≥180 mg/dl, Two hours: ≥153 mg/dl. Preeclampsia as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation, with proteinuria (≥ 300 mg protein in 24-hour urine, or result of at least +1 in dipstick) in women with normal blood pressure before pregnancy. Also, for this purpose, blood pressure was measured at least two times with a 6-hour interval.

SPSS software version 22 (SPSS Inc., Chicago, IL, U.S.A.) used for statistical. The collected data were described by mean and standard deviation or frequency and percentage. To investigate the differences between the two groups and compare the quantitative and qualitative variables, student t-test and Chi-square tests were used, respectively. The significance level in the tests was considered at 0.05.

Ethical considerations

This study was approved by the Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Ethic Code: IR.AJUMS.HGOLESTAN.REC.1399.077.

The expense of this thesis has been provided by the credit of the approved research project no. FIRC-9918 (Approval Date: 20-9-2020) in Ahvaz Jundishapur University of Medical Sciences.

Informed and written consent was taken from all patients before entering the research. In addition, in all stages of this study, the provisions of the ethics statement in Helsinki research and the principles of patient information confidentiality were observed. All women with abnormal TSH levels were referred to an endocrinologist for further evaluation and treatment. Patients were not charged for the tests. The costs of the tests were funded by Ahvaz Jundishapur University of Medical Sciences.

Results

The present study was conducted on 448 pregnant women with mean ± S.D age of 31.63±5.00 years. A diagram of the research process is presented in Figure 1.

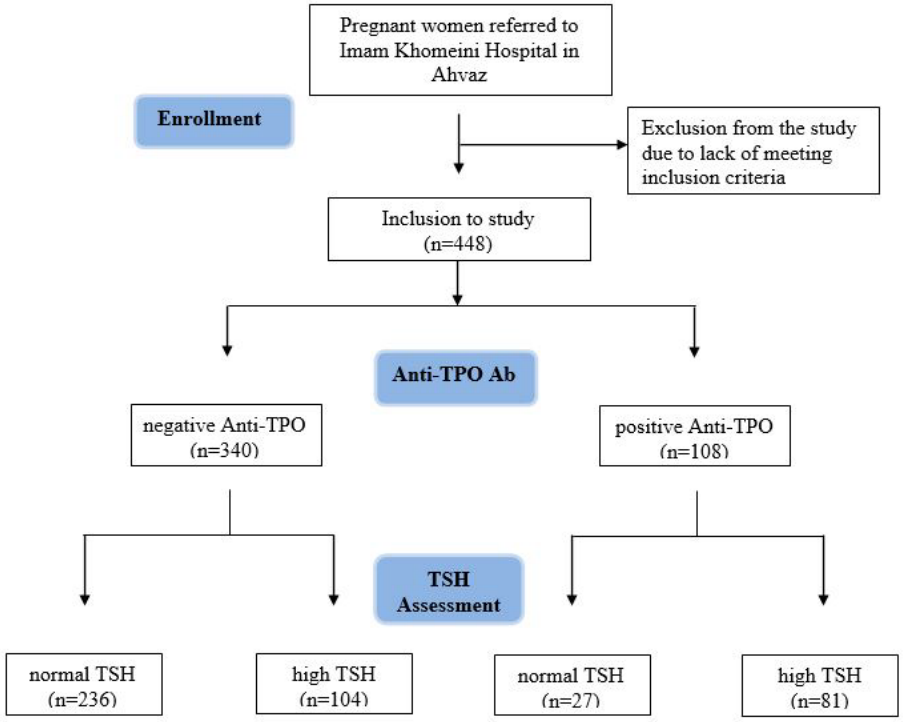


Figure 1. Diagram of Research Process

A positive anti-peroxidase antibody was seen in 108 patients (24.10%), and a negative anti-peroxidase antibody was observed in 340 patients (75.90%). The results of comparing the basic characteristics of patients in two groups of positive and negative anti-TPO are presented in Table 1.

Table 1. Comparison of underlying characteristics of women in two groups of positive TPO Ab and negative TPO Ab.

Variable	TPO Ab positive (n=108)	Negative TPO Ab (n=340)	P-value*
Age (years), mean (S.D), (min, max)	31.64 (5.10) (19,35)	31.54 (5.02) (18,35)	0.865
Number of pregnancies, n (%)	2.23 (1.48) (1,9)	2.25 (1.31) (1,7)	0.902
Number of deliveries, n (%)	0.75 (0.82) (0,5)	0.82 (0.90) (0,4)	0.488
Number of miscarriages, n (%)	0.49 (1.03) (0,7)	0.42 (0.77) (0,4)	0.454
Number of live children, n (%)	0.69 (0.77) (0,4)	0.74 (0.82) (0,4)	0.622
BMI (kg/m^2), mean (S.D), (min, max)	26.72 (4.33) (17,40)	26.76 (4.52) (17,45)	0.946

Variable	TPO Ab positive (n=108)	Negative TPO Ab (n=340)	P-value*
History of delivery			
No history of delivery, n (%)	46 (42.59)	152 (44.70)	0.837
normal delivery, n (%)	5 (4.62)	33 (9.70)	
Cesarean section, n (%)	55 (50.92)	147 (53.23)	
Both, n (%)	2 (1.85)	8 (2.35)	
TSH (µIU/ml), mean (S.D), (min, max)	4.82 (9.38) (0.01,96.0)	2.25 (1.47) (0.01,8.10)	<0.0001*
high TSH, n (%)	81 (75.00)	104 (30.58)	<0.0001*
Normal TSH, n (%)	27 (25.00)	236 (69.41)	

Abbreviations: BMI: Body mass index; TSH: thyroid-stimulating hormone
*** Significant differences at level of 0.05**

There was no significant difference between the two groups in terms of age, number of pregnancies, number of previous deliveries, number of abortions, number of live children and body mass index (BMI).

The results of t-test showed that TSH level in positive TPO Ab women was significantly higher than that in negative TPO Ab women (P<0.0001). The frequency of high TSH in positive TPO Ab women was significantly higher in comparison to negative TPO Ab women (P<0.0001).

The relationship between the level of positive TPO Ab and disease in pregnancy and the negative outcome of pregnancy is presented in Table 2.

Table 2. Association between positive TPO Ab level and negative outcome of pregnancy.

Outcomes	TPO Ab positive (n=108)	Negative TPO Ab (n=340)	P-value
	N (%)	N (%)	
Gestational Diabetes	50 (46.29)	116 (34.11)	0.029*
Preeclampsia	12 (11.11)	34 (10.00)	0.719
Miscarriage	17 (15.74)	14 (4.11)	<0.0001*
Preterm delivery	15 (13.88)	12 (3.52)	<0.0001*
IUGR evidence	7 (6.48)	12 (3.52)	0.355

IUGR: Intrauterine growth restriction
*** Significant differences at level of 0.05**

The frequency of GDM in positive anti-TPO Ab pregnant women is significantly higher than in the negative anti-TPO Ab group (P=0.029). No significant difference was recognized between the two groups regarding incidence of preeclampsia (P=0.719). The miscarriage and preterm delivery frequency in the positive TPO Ab group were significantly higher than that in the negative TPO Ab group (P<0.001). The frequency of IUGR based on ultrasound findings was (P=0.335) and no significant difference between two groups. Placental abruption and IUFD were not seen in any of the two groups.

The results of investigating the association between the adverse outcomes in euthyroid women based on positive or negative TPO Ab are presented in Table 3.

Table 3. Frequency of negative outcomes of pregnancy in euthyroid women based on TPO Ab.

Outcomes	Negative TPO (n=236)	Positive TPO (n=27)	P-value
	N (%)	N (%)	
Miscarriage	0 (0)	1 (3.70)	0.041*
Preterm delivery	1 (0.42)	1 (3.70)	0.048*
Gestational Diabetes	27 (11.44)	6 (22.22)	0.003*
Preeclampsia	14 (5.93)	2 (7.40)	0.034*

*** Significant differences at level of 0.05**

The adverse pregnancy outcomes in positive anti-TPO euthyroid women are higher than those in the negative ($P<0.05$).

Discussion

In this study, the rate of adverse pregnancy outcomes including GDM, miscarriage and preterm delivery in the positive TPO ab group, was significantly higher than that in the negative TPO Ab group, but the frequency of IUGR was not significantly different between the two groups.

The results of several studies have confirmed a statistically significant relationship between thyroid antibodies and the risk of GDM **(12-14)**.

In a research done by Gupta et al., preterm birth, miscarriage, and other maternal and fetal outcomes were high in anti-TPO positive women **(15, 16)**. Thus, the results suggest that anti-TPO Ab can be one of the markers of increased miscarriage.

In research carried out by Negro et al. in Italy, preterm delivery was significantly higher in the positive anti-TPO Ab group than in the negative anti-TPO group, (22.4% vs. 8.2%) **(17)**. In a research, preterm delivery in the positive anti-TPO Ab group was significantly more than negative anti-TPO group (16.1% vs. 7.9%) **(18, 19)**. Ghafoor, Mansoor **(20)** also reported that the rate of preterm delivery in the positive anti-TPO Ab women was significantly higher than that of negative anti-TPO Ab women (26.8% vs. 8%) **(20)**. In research conducted by Rajput, Yadav **(8)**, miscarriage was significantly higher in the positive TPO Ab group than in the negative (12% vs. 3.3%). Premature birth was also higher in the positive TPO Ab women (14% vs. 3.3%). In another study by Taha **(21)**, pregnant women with overt hypothyroidism with positive anti-TPO had higher TSH levels than negative anti-TPO women and the prevalence of adverse pregnancy outcomes, including GDM, IUFD, and miscarriage was higher in positive anti-TPO women. In a research done by Abbassi-Ghanavati, Casey **(22)**, a threefold enhancement in miscarriage was related in the positive anti-TPO Ab group in comparison to the negative anti-TPO Ab group (1% vs. 0.3%). Two meta-analysis studies also reported that positive anti-TPO Ab pregnant women had a higher risk of preterm parturition than negative anti-TPO Ab women **(23, 24)**. The pathophysiological mechanism of preterm birth in autoantibody thyroid has not yet been clarified **(24)**.

On the other hand, Yuan, Sun **(25)** showed that positive anti-TPO Ab in euthyroid women was not related to negative expectancy results such as GDM, gestational hypertension, spontaneous miscarriage, low birth weight, and preterm birth. However, anti-TPO Ab in women with female fetuses was significantly associated with preterm birth. Tierney, Delpachitra **(26)** and Chen, Jin **(11)** indicated no significant relationship between preterm delivery in the two groups of negative anti-TPO Ab and positive anti-TPO Ab women. Bhat, AP **(1)** showed that the risk of miscarriage, gestational hypertension, and GDM was not significantly different between positive TPO Ab and negative TPO Ab group. The results of this study contradicted our findings .Some differences in the results of different studies can be attributed to differences in methodology, sample size, and characteristics of the study population. In addition, the existence of inconsistencies in the results of studies prevents definitive conclusions about the relationship between positive TPO Ab and adverse outcomes of pregnancy, and there is a need for further investigations in this regard.

In this study, the TSH level and frequency of hypothyroidism in positive TPO Ab women was significantly higher than the negative TPO Ab group. In the research done by Rajput, Yadav **(8)**, the TSH levels in positive TPO Ab pregnant women were significantly higher than that in the negative TPO Ab group. Yuan, Sun **(25)** in China also showed that TSH levels were significantly higher in the positive anti-TPO Ab group than the negative anti-TPO Ab group **(25)**. Increased TSH levels in positive TPO Ab women may be due to immune-mediated inflammation in the thyroid gland, leading to reduced thyroid function, related to decreased thyroid adaptation to physiological changes in pregnancy **(18)**.

The present study and similar studies show that the adverse outcomes of pregnancy in positive TPO Ab women are more than those of negative anti-TPO women, and in the case of high levels of TSH, adverse outcomes of pregnancy increase more. However, the association between positive TPO Ab in expectant women and the adverse pregnancy outcomes has not been fully identified, and the underlying mechanisms included in this relationship have not been identified. Thyroid autoantibodies can have a negative effect on pregnancy in TSH-dependent or non-TSH-dependent ways. Since various studies have indicated that baseline TSH levels are significantly higher in positive TPO Ab pregnant women than negative **(27)**, it seems that women with positive TPO Ab before expectancy may have thyroid dysfunction, which may be exacerbated during pregnancy **(28)** and subclinical or overt hypothyroidism may occur during pregnancy **(28-30)**. Finally, these results suggest that apart from the underlying mechanism of association between positive TPO Ab and TSH levels, timely treatment of hypothyroidism is crucial in reducing the adverse outcomes of pregnancy.

There were some limitations in this study. It was a single-center study, sample selection bias was unavoidable and some demographic characteristics like socioeconomic status, level of education and nutritional status, and iodine consumption were not considered. It also investigated only the effects of positive anti-TPO Ab on several adverse pregnancy outcomes. It did not investigate some outcomes such as neonates' birth weight and LBW.

Conclusion

The present study results showed that there was a critical relationship between the presence of positive anti-TPO Ab and negative expectancy results such as preterm parturition, miscarriage, and GDM. Due to the high outbreak of thyroid disorders and their relationship with adverse pregnancy outcomes, initial screening of thyroid abnormalities and routine TPO Ab testing during pregnancy, and timely treatment of thyroid disorders are recommended to prevent adverse pregnancy outcomes.

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This study was approved by the Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences, with Ethic Code: IR.AJUMS.HGOLESTAN.REC.1399.077. We thank the coordinators and data collectors who assisted in this study.

Authors' Contributions

Nahid Shahbazian: Conceived and designed the analysis; Contributed data or analysis tools; wrote the paper. Mojgan Barati: Conceived and designed the analysis; wrote the paper. Mahvash Zargar: Conceived and designed the analysis; wrote the paper. Najmieh Saadati: Conceived and designed the analysis; wrote the paper. Elham Direkvand: Conceived and designed the analysis; collected the data; contributed data or analysis tools; performed the analysis; wrote the paper.

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Conflict of Interest

The authors declare no conflict of interest.

Funding

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