

Thyroid Peroxidase Antibody Positivity in Early Pregnancy and Its Association with Recurrent Pregnancy Loss, Obstetric, and Perinatal Outcomes: A Prospective Observational Study

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Abstract

Background: Thyroid peroxidase antibodies (TPOAb) are markers of thyroid autoimmunity associated with adverse pregnancy outcomes.

Objective: To determine the prevalence of TPOAb positivity and its association with recurrent pregnancy loss (RPL), maternal, and perinatal outcomes.

Methods: This prospective observational study included 100 pregnant women with gestational age ≤ 20 weeks. Thyroid function tests and anti-TPO antibody levels were assessed, and participants were followed until delivery. Maternal and perinatal outcomes were recorded and analyzed.

Results: TPOAb positivity was observed in 32% of participants. TPOAb-positive women had significantly higher TSH levels ($p < 0.001$) and a higher prevalence of RPL (56.3% vs. 17.6%, $p < 0.001$). Significant associations were observed with gestational hypertension ($p = 0.042$), preeclampsia ($p = 0.037$), preterm labour ($p = 0.047$), low birth weight ($p = 0.021$), and NICU admission ($p = 0.048$). Logistic regression identified TPOAb positivity (OR=3.46, $p = 0.006$) and subclinical hypothyroidism (OR=2.63, $p = 0.033$) as independent predictors of RPL.

Conclusion: TPOAb positivity is common in pregnancy and is significantly associated with recurrent pregnancy loss and adverse maternal and perinatal outcomes. Early screening may help identify high-risk pregnancies and improve outcomes.

Keywords: Thyroid Peroxidase Antibodies; Thyroid Autoimmunity; Recurrent Pregnancy Loss; Subclinical Hypothyroidism; Pregnancy Outcomes.

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Introduction

The thyroid gland plays a crucial role in regulating metabolism, growth, and development through the secretion of thyroxine (T4) and triiodothyronine (T3). [1] Pregnancy induces significant physiological changes in thyroid function, resulting in approximately a 50% increase in thyroid hormone production and an increased requirement for iodine to meet maternal and fetal demands. [2] Human chorionic gonadotropin (hCG) stimulates thyroid hormone synthesis during early pregnancy, while elevated estrogen levels increase thyroid-binding globulin concentrations, leading to

alterations in thyroid hormone metabolism and regulation. [3] Adequate maternal thyroid function is essential for normal fetal growth and neurodevelopment, particularly during the first trimester when the fetus is entirely dependent on maternal thyroid hormones. [4] Thyroid disorders are among the most common endocrine abnormalities encountered during pregnancy. These disorders range from subclinical hypothyroidism to overt thyroid dysfunction and are associated with adverse maternal and fetal outcomes. [5] A recent meta-analysis reported a pooled prevalence of

hypothyroidism among pregnant women of 11.07%, with subclinical hypothyroidism accounting for 9.51% and overt hypothyroidism for 2.74%. [6] Untreated thyroid dysfunction has been linked to miscarriage, preeclampsia, preterm birth, impaired fetal neurodevelopment, and increased perinatal morbidity. [7]

Autoimmune thyroid disease (AITD), particularly Hashimoto's thyroiditis, represents the most common cause of hypothyroidism in women of reproductive age. Thyroid autoantibodies, especially thyroid peroxidase antibodies (TPOAb), serve as sensitive markers of thyroid autoimmunity and may be present even in euthyroid women. TPO antibodies are detected in approximately 5–20% of women of reproductive age and are associated with an increased risk of developing hypothyroidism during pregnancy and the postpartum period. [8-10]

Thyroid peroxidase is a membrane-bound enzyme responsible for the oxidation and organification of iodide during thyroid hormone synthesis. Autoantibodies directed against thyroid peroxidase result from an autoimmune loss of immune tolerance and contribute to thyroid gland inflammation and dysfunction. TPO antibodies are recognized as the most sensitive and specific serological marker for autoimmune thyroiditis, being present in 90–95% of affected individuals. [10-12]

Several studies have demonstrated that TPO antibody positivity is associated with adverse obstetric and perinatal outcomes, even in women with normal thyroid hormone levels. TPO-positive women have a two- to four-fold increased risk of miscarriage and are more likely to experience preterm delivery, gestational hypertension, preeclampsia, gestational diabetes mellitus, low birth weight, and neonatal intensive care unit (NICU) admission. [13,14] Furthermore, maternal thyroid dysfunction has been associated with increased risks of preterm birth, low Apgar scores, postpartum hemorrhage, and adverse neonatal outcomes. [15,16]

Despite growing evidence linking thyroid autoimmunity with unfavorable pregnancy outcomes, considerable variations exist across studies due to differences in population characteristics, iodine status, screening practices, and diagnostic criteria. Moreover, region-specific data from central India, particularly Madhya Pradesh, remain limited. Early identification of TPO antibody-positive pregnant women, especially before 20 weeks of gestation, may facilitate timely monitoring and intervention, thereby reducing the risk of maternal and neonatal complications. [17,18]

Therefore, the present study was undertaken to evaluate the prevalence of thyroid peroxidase antibody positivity among pregnant women with gestational age less than 20 weeks and to assess its association with obstetric and perinatal outcomes in a tertiary care centre. The objective of this study was to determine the prevalence of TPOAb positivity and evaluate its association with recurrent pregnancy loss and adverse maternal and perinatal outcomes.

Materials and Methods

This prospective observational study was conducted in the Department of Obstetrics and Gynaecology in collaboration with the Department of Endocrinology at People's College of Medical Sciences and Research Centre, Bhopal, India. The study was approved by the Institutional Ethics Committee (IEC Approval No. PCMS/OD/PS/2 024/73877), and written informed consent was obtained from all participants.

The sample size was calculated based on an anticipated TPOAb positivity prevalence of 20%, with 95% confidence level and 8% absolute precision. The minimum required sample size was estimated to be 97, which was rounded to 100 participants. Pregnant women with singleton intrauterine pregnancies of ≤ 20 weeks' gestation attending the antenatal outpatient or inpatient services and referred for thyroid evaluation were screened for eligibility. Women willing to participate and provide written informed consent were included in the study. Women with gestational age > 20 weeks, multifetal pregnancy, molar pregnancy, known hyperthyroidism, or refusal to provide consent were excluded. All eligible women presenting during the study period were consecutively enrolled.

At enrollment, detailed demographic and clinical information including age, obstetric history, medical history, height, weight, blood pressure, hemoglobin level, and random blood sugar was recorded using a structured proforma. A comprehensive clinical examination was performed, including assessment for pallor and thyroid enlargement (goiter).

Venous blood samples were collected from all participants for thyroid function assessment. Serum free triiodothyronine (fT3), free thyroxine (fT4), and thyroid-stimulating hormone (TSH) levels were measured using electrochemiluminescence immunoassay (ECLIA) with commercially available assay kits. Anti-thyroid peroxidase (anti-TPO) antibody levels were determined using a solid-phase, enzyme-labelled chemiluminescent sequential immunometric assay. Anti-TPO antibody levels ≥ 35 IU/mL were considered positive, whereas levels < 35 IU/mL were

considered negative. Thyroid status was classified according to the American Thyroid Association guidelines. Euthyroidism was defined as normal fT4 levels with trimester-specific TSH values (≤ 2.5 mIU/L in the first trimester and ≤ 3.0 mIU/L in the second trimester). Subclinical hypothyroidism was defined as elevated TSH with normal fT4 levels, whereas overt hypothyroidism was defined as elevated TSH associated with reduced fT4 levels.

Participants were prospectively followed throughout pregnancy until delivery. Maternal outcomes assessed included preeclampsia, gestational diabetes mellitus (GDM), gestational hypertension, preterm labour, recurrent abortion, and mode of delivery. Perinatal outcomes evaluated were gestational age at delivery, birth weight, low birth weight (< 2.5 kg), neonatal intensive care unit (NICU) admission, need for ventilatory support, and other neonatal complications. Preeclampsia was defined as new-onset hypertension (blood pressure $\geq 140/90$ mmHg) with proteinuria after 20 weeks of gestation, while preterm birth was defined as delivery before 37 completed weeks of gestation.

All laboratory investigations were performed in the institutional central laboratory using standardized protocols and calibrated equipment. Internal quality-control procedures were routinely followed to ensure analytical accuracy. Participant confidentiality was maintained by assigning unique identification numbers and restricting data access to the investigators.

Data was analyzed using IBM SPSS Statistics version 30.0 (IBM Corp., Armonk, NY, USA). Comparisons between groups were performed using the independent samples t-test, Chi-square test. Pearson's correlation analysis was used to assess the relationship between anti-TPO antibody levels and thyroid function parameters, while logistic regression analysis was performed to identify independent predictors of adverse pregnancy outcomes. A p-value < 0.05 was considered statistically significant.

Results

The mean age of the participants was 27.9 ± 4.8 years. Most women were from urban areas (58%), while 42% belonged to rural areas. Multigravida women constituted 64% of the study population, whereas 36% were primigravida.

A history of recurrent pregnancy loss (two or more previous pregnancy losses) was present in 30% of participants. The mean height and weight were 156.8 ± 6.4 cm and 62.5 ± 9.3 kg, respectively. The mean hemoglobin level was 11.2 ± 1.4 g/dL, and the mean random blood sugar level was 96.3 ± 14.7

mg/dL. The mean serum TSH level of the study participants was 3.12 ± 1.68 mIU/L, while the mean fT3 and fT4 levels were 3.41 ± 0.56 pg/mL and 1.32 ± 0.28 ng/dL, respectively.

The mean anti-TPO antibody level was 124.6 ± 210.4 IU/mL, indicating considerable variability in thyroid autoimmunity among participants. TPOAb positivity was observed in 32% of pregnant women, whereas 68% were TPOAb negative. Based on thyroid function assessment, 54% of women were euthyroid, 38% had subclinical hypothyroidism, and 8% had overt hypothyroidism. These findings demonstrate a substantial burden of thyroid autoimmunity and thyroid dysfunction in the study population, with subclinical hypothyroidism being the most common thyroid abnormality.

Baseline demographic and clinical characteristics were comparable between TPOAb-positive and TPOAb-negative women ($p > 0.05$). However, TPOAb-positive women had significantly higher mean TSH levels than TPOAb-negative women (4.82 ± 2.16 vs. 2.71 ± 1.14 mIU/L, $p < 0.001$).

No significant differences were observed in fT3 or fT4 levels between the groups, suggesting an association between TPOAb positivity and elevated TSH levels. (Table 1) TPOAb-positive women demonstrated significantly higher rates of recurrent pregnancy loss, gestational hypertension, preeclampsia, and preterm labour. No significant association was observed with gestational diabetes mellitus. (Table 2) TPOAb positivity was significantly associated with low birth weight and NICU admission. Although stillbirth and neonatal death were more frequent among TPOAb-positive women, these associations did not reach statistical significance. (Table 3) The incidence of adverse pregnancy outcomes increased progressively from euthyroid women to those with subclinical and overt hypothyroidism.

Women with overt hypothyroidism demonstrated the highest rates of preeclampsia, preterm labour, and low birth weight. (Table 4) Anti-TPO antibody levels showed a moderate positive correlation with TSH and a weak negative correlation with fT4. Higher maternal TSH levels were significantly associated with lower neonatal birth weight. (Table 5) Multivariate analysis identified TPOAb positivity and subclinical hypothyroidism as independent predictors of adverse pregnancy outcomes. TPOAb-positive women had approximately three-fold higher odds of adverse outcomes compared with TPOAb-negative women. (Table 6)

Table 1. Comparison of Baseline Characteristics and Thyroid Parameters Between TPOAb-Positive and TPOAb-Negative Women (n=100)

Variable	TPOAb Positive (Mean $\pm$ SD) (n=32)	TPOAb Negative (Mean $\pm$ SD) (n=68)	t-value	p-value
Age (years)	29.1 $\pm$ 4.6	27.3 $\pm$ 4.8	1.80	0.071
Weight (kg)	63.4 $\pm$ 8.6	61.8 $\pm$ 9.7	0.83	0.414
Hemoglobin (g/dL)	10.9 $\pm$ 1.3	11.3 $\pm$ 1.5	-1.36	0.183
Random blood sugar (mg/dL)	98.1 $\pm$ 13.9	95.4 $\pm$ 15.2	0.88	0.374
TSH (mIU/L)	4.82 $\pm$ 2.16	2.71 $\pm$ 1.14	5.20	0.001*
fT3 (pg/mL)	3.28 $\pm$ 0.54	3.48 $\pm$ 0.57	-1.70	0.091
fT4 (ng/dL)	1.26 $\pm$ 0.25	1.35 $\pm$ 0.29	-1.59	0.118

*Statistically significant

Table 2. Association Between TPO Antibody Positivity and Maternal Outcomes (TPO)(n=100)

Outcome	TPOAb Positive (n=32) n (%)	TPOAb Negative (n=68) n (%)	χ^2 value	p-value
Recurrent pregnancy loss	18 (56.3)	12 (17.6)	15.44	<0.001*
Gestational hypertension	9 (28.1)	8 (11.8)	4.13	0.042*
Preeclampsia	7 (21.8)	5 (7.3)	4.35	0.037*
Gestational diabetes mellitus	6 (18.7)	9 (13.2)	0.52	0.471
Preterm labour	10 (31.2)	11 (16.1)	3.94†	0.047*

*Statistically significant

Table 3. Association between TPO Antibody Positivity and Perinatal Outcomes (n=100)

Outcome	TPOAb Positive (n=32) n (%)	TPOAb Negative (n=68) n (%)	χ^2 value	p-value
Low birth weight	14 (43.7)	14 (20.6)	5.35	0.021*
NICU admission	9 (28.1)	10 (14.7)	3.90	0.048*
Stillbirth	4 (12.5)	3 (4.4)	1.95	0.163
Neonatal death	2 (6.2)	1 (1.5)	1.50	0.221

*Statistically significant

Table 4. Pregnancy Outcomes According to Thyroid Status (N=100)

Outcome	Euthyroid (n=54) n (%)	Subclinical Hypothyroidism (n=38) n (%)	Overt Hypothyroidism (n=8) n (%)	χ^2 value	p-value
Preeclampsia	4 (7.4)	6 (15.8)	2 (25.0)	6.39	0.041*
Preterm labour	7 (13.0)	11 (28.9)	3 (37.5)	6.89	0.032*
Low birth weight	10 (18.5)	14 (36.8)	4 (50.0)	7.17	0.028*

Table 5. Correlation between Anti-TPO Antibody Levels, Thyroid Parameters, and Birth Weight

Variable	Correlation Coefficient (r)	p-value
Anti-TPO vs TSH	0.52	<0.001*
Anti-TPO vs fT3	-0.14	0.162
Anti-TPO vs fT4	-0.21	0.038*
TSH vs Birth Weight	-0.29	0.004*

Table 6. Multivariate Logistic Regression Analysis for Adverse Pregnancy Outcomes

Variable	Adjusted OR	95% CI	p-value
TPOAb positivity	3.12	1.26–7.70	0.014*
Subclinical hypothyroidism	2.41	1.02–5.66	0.044*
Maternal age >30 years	1.58	0.64–3.88	0.315

Discussion: The study demonstrated a high prevalence of TPOAb positivity (32%), with significant associations between thyroid autoimmunity and recurrent pregnancy loss (RPL), hypertensive disorders of pregnancy, preterm

labour, low birth weight, and neonatal intensive care unit (NICU) admission. In the present study, 32% of pregnant women were positive for TPO antibodies. This prevalence is higher than that reported in most international studies, where

TPOAb positivity ranges from 5% to 20% among women of reproductive age. [8,10] Similar findings reported by Mahadik et al. [19] reported lower prevalence rates, ranging from 10% to 20%. The relatively higher prevalence observed in the present study may be attributed to regional variations in iodine intake, genetic susceptibility, and the inclusion of women with thyroid dysfunction, particularly subclinical hypothyroidism. Previous studies from South Asian populations have similarly reported higher frequencies of thyroid autoimmunity compared with Western populations. [20]

A major finding of this study was the significant association between TPOAb positivity and recurrent pregnancy loss. Women with positive TPO antibodies had a 3.46-fold higher risk of RPL, and TPOAb positivity emerged as an independent predictor on multivariable analysis. These findings are consistent with the meta-analysis by Thangaratinam et al., [21] which demonstrated a nearly three-fold increased risk of miscarriage among women with thyroid autoantibodies. Similarly, Negro et al. [22] reported significantly higher miscarriage rates among untreated TPOAb-positive women compared with antibody-negative controls. The association between thyroid autoimmunity and pregnancy loss may be explained by immune dysregulation, impaired placental development, increased natural killer cell activity, and subtle thyroid hormone insufficiency affecting early fetal development. [23,24]

The present study also found significantly higher rates of gestational hypertension, preeclampsia, and preterm labour among TPOAb-positive women. These observations are in agreement with previous reports demonstrating an association between thyroid autoimmunity and adverse maternal outcomes. Hu et al. [25] reported that TPOAb positivity was associated with an increased risk of hypertensive disorders during pregnancy, Chronic inflammation, endothelial dysfunction, and impaired placental vascular development associated with thyroid autoimmunity may contribute to these complications. In contrast, no significant association was observed between TPOAb positivity and gestational diabetes mellitus, which is consistent with previous studies suggesting a weak or inconsistent relationship between thyroid autoimmunity and glucose metabolism. [26]

With respect to perinatal outcomes, TPOAb-positive women had significantly higher rates of low birth weight and NICU admission. Furthermore, maternal TSH levels demonstrated a significant negative correlation with neonatal birth weight, indicating that increasing thyroid dysfunction may adversely affect fetal growth. These findings are supported by previous studies that reported an association between thyroid

dysfunction, placental insufficiency, fetal growth restriction, and neonatal morbidity. [27] Although stillbirth and neonatal death occurred more frequently among TPOAb-positive women, these differences were not statistically significant, likely because of the relatively small sample size and limited number of events.

Another important observation was the significant association between thyroid autoimmunity and thyroid dysfunction. TPOAb-positive women exhibited significantly higher TSH levels, and anti-TPO antibody levels showed a moderate positive correlation with serum TSH concentrations. These findings support the established role of TPO antibodies as markers of autoimmune thyroid disease and predictors of progression from euthyroidism to overt hypothyroidism. [28] The autoimmune destruction of thyroid tissue leads to reduced thyroid reserve, making affected women more vulnerable to thyroid insufficiency during pregnancy when hormonal demands are increased.

The present study further demonstrated a progressive increase in adverse pregnancy outcomes from euthyroid women to those with subclinical and overt hypothyroidism. The incidences of preeclampsia, preterm labour, and low birth weight were highest among women with overt hypothyroidism. Similar observations have been reported by Eshkoli et al., [29] who showed that increasing severity of thyroid dysfunction was associated with worsening maternal and fetal outcomes. Reduced thyroid hormone availability may impair placental development, uteroplacental circulation, and fetal growth, thereby contributing to adverse obstetric outcomes.

The findings of this study have important clinical implications. Early assessment of thyroid function and TPO antibody status during the first half of pregnancy may help identify women at increased risk of adverse maternal and neonatal outcomes. Screening for thyroid autoimmunity, particularly among women with subclinical hypothyroidism or a history of pregnancy loss, may facilitate timely intervention and closer antenatal surveillance. The observed associations also support current recommendations advocating appropriate monitoring and treatment of thyroid dysfunction during pregnancy. [7,18] The study has certain limitations. It was conducted at a single tertiary care centre and included a relatively modest sample size, which may limit generalizability and reduce statistical power for uncommon outcomes such as stillbirth and neonatal death. In addition, potential confounding factors such as iodine status, dietary intake, and socioeconomic variables were not assessed. Nevertheless, the prospective design, systematic follow-up, and comprehensive evaluation of both maternal and perinatal outcomes strengthen the validity of the findings.

Conclusion: TPOAb positivity was present in nearly one-third of pregnant women and was independently associated with recurrent pregnancy loss, hypertensive disorders, preterm labour, low birth weight, and NICU admission.

Early screening for thyroid autoimmunity and appropriate management may help identify high-risk pregnancies and improve maternal and neonatal outcomes.

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