ISSN Print: 2664-8393
ISSN Online: 2664-8407
IJGS 2025; 7(2): 01-06
www.gynaecologyjournal.net
Received: 08-03-2025
Accepted: 12-04-2025

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Thyroglobulin antibodies and their role alongside thyroid-stimulating hormone in abnormally invasive placenta

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DOI: https://www.doi.org/10.33545/26648393.2025.v7.i2a.45

Abstract
Background: Abnormally Invasive Placenta (AIP), encompassing *placenta accreta*, increta, and percreta, results from excessive trophoblastic invasion beyond the decidual barrier. This pathological process is associated with disrupted angiogenesis, elevated vascular endothelial growth factors, and altered immune responses. While multiple biomarkers have been investigated for predicting AIP, their diagnostic accuracy remains controversial. Recent evidence suggests a potential role for thyroid function markers including Thyroid Stimulating Hormone (TSH) and thyroglobulin antibodies (TgAb) in modulating placental invasion and vascular remodeling.
Objective: To investigate the relationship between maternal thyroid function specifically serum levels of TSH and TgAb and the incidence and severity of AIP in pregnant women.
Methods: This prospective case-control study included 105 third-trimester pregnant women at Tikrit Teaching Hospital, Iraq (November 2024 to June 2025). Participants were divided into three equal groups (N=35 each): AIP group (confirmed intraoperatively and histologically), placenta previa totalis (PPT) group without invasion, and healthy controls. Exclusion criteria included thyroid disease, autoimmune disorders, and other confounding obstetric or medical conditions. Thyroid markers (TSH, T_3, T_4, TgAb, TPOAb) were assessed via electrochemiluminescence. Diagnostic accuracy was evaluated using ROC analysis. Obstetric and neonatal outcomes were compared across biomarker levels.
Results: Women with AIP had significantly lower TSH and higher T_4 levels compared to PPT and controls ($p<0.05$). ROC analysis identified a TSH cutoff of 2.25 mIU/L with 74.29% sensitivity and 70.00% specificity (AUC=0.831, $p<0.001$) for predicting AIP. Logistic regression showed TSH (OR=0.36, P=0.03) and TgAb (OR=0.68, P=0.04) as significant predictors. Lower TSH and higher TgAb levels were associated with reduced risks of cesarean hysterectomy and massive transfusion. High TSH was linked to adverse neonatal outcomes including preterm birth (62.86%) and NICU admission (51.43%).
Conclusion: Maternal thyroid function-particularly elevated TSH and low TgAb levels is associated with increased risk and severity of AIP and poorer maternal-neonatal outcomes. TSH and TgAb may serve as useful biomarkers in the early identification and risk stratification of AIP in pregnant women. Further large-scale studies are warranted to validate these findings and explore underlying mechanisms.

Keywords: AIP, encompassing *placenta accreta*, increta, mechanisms

1. Introduction
Placentation is a complex process that requires extravillous trophoblasts (EVT) to move. Interstitial trophoblasts enter the decidua of the mother, and endovascular trophoblasts move into the mother's spiral arteries. When a woman is pregnant and healthy, the protective layer of the decidua acts as a balance between trophoblastic invasion and angiogenesis. When a placenta is abnormally invasive (AIP), trophoblastic invasion goes beyond the decidual layer. This shows up as a range of stages: *placenta accreta*, where the placenta attaches to the myometrium; placenta increta, where trophoblasts invade the myometrium and placenta percreta, where the placenta invades through the myometrium, serosa, and nearby structures [2]. AIP is thought to be caused by too much trophoblast invasion and decidual failure. Cytokines that encourage invasion, such as interleukin (IL)-33 and IL-6, have been found to be higher in placenta accrete [3, 4].

Beyond the disruption of the tightly regulated physiological mechanisms, another critical factor in cases of abnormally invasive placenta (AIP) is the extensive uteroplacental neovascularization observed in areas of placental adherence. Studies have indicated that the extravillous trophoblast (EVT) invasion associated with *placenta accreta* correlates with altered levels of angiogenic and growth-related molecules [5]. Notably, concentrations of angiogenic factors such as vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang2) are elevated in *placenta accreta* cases, whereas levels of the anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) are significantly lower compared to normal placentas [6]. Although multiple biomarkers have been proposed, the predictive accuracy for AIP among patients with placenta previa totalis (PPT) remains controversial.

Thyroid hormones (TH) and thyroglobulin antibodies (TgAb) have been recognized as vital for reproductive function [7]. Both TH and TgAb are implicated in early placental formation, fetal development, and are associated with pregnancy complications like miscarriage and preterm delivery [9]. Trophoblast cells express both thyroid hormone transporters and receptors, and adequate TH levels are essential for successful placentation, partly through the regulation of growth factors and cytokines that facilitate EVT invasion and the vascular remodeling of maternal-fetal circulation [10].

Additionally, TgAbs may enhance the development and activity of maternal and fetal thyroid tissues until the fetal thyroid matures during late gestation. Thyroid-stimulating hormone (TSH), in particular, is thought to impact vascular endothelial proliferation by supporting angiogenic processes, leukocyte adhesion, and exerting anti-inflammatory effects. Elevated maternal TSH concentrations have been observed in preterm births linked with vascular disorders, such as preeclampsia, suggesting that increased TSH might contribute to inadequate trophoblastic invasion [11, 12].

Despite numerous suggestions about the involvement of angiogenic and anti-inflammatory factors in aberrant trophoblastic invasion, the precise etiology remains ambiguous. We anticipated that maternal TH, TSH, and anti-thyroid antibody levels may influence trophoblastic invasion and angiogenesis in AIP, as indicated by the aforementioned studies.

2. Methods

2.1 Data collection and clinical evaluation

During the procedure of enrollment, comprehensive histories in obstetrics, surgery, and medicine were obtained for each subject by the review of medical records with structured interviews. A standardized case report form was used to record all relevant clinical and demographic information consistently. Maternal age and BMI were recorded at admission. History of previous cesarean sections and any uterine curettages was also noted. Gestational age was recorded both at the time of blood sampling in the third trimester and at delivery, based on the last menstrual period and confirmed by early ultrasound. In addition, neonatal outcomes such as birth weight and Apgar scoring measures of 1 and 5 minutes were recorded. The mode of delivery and detailed intraoperative findings, especially concerning placental location and adhering, were noted in each case to aid with diagnostic classification.

2.2 Laboratory investigations

During the early third trimester (between 29 and 31 weeks gestation), the whole blood was collected following overnight fasting. The specimens were obtained by venipuncture from the antecubital brachial vein, placed in plain tubes, and centrifuged to separate serum. The markers measured were:

- Thyroid-Stimulating Hormone (TSH)
- Triiodothyronine (T_3)
- Thyroxine (T_4)
- Thyroglobulin Antibodies (TgAb)
- Thyroid Peroxidase Antibodies (TPOAb)

The tests were done by the Electrochemiluminescence Immunoassay (ECLIA) technique on the Roche Elecsys 2010 system. Further blood samples were again taken at delivery for reassessment of TSH, T_3, and T_4.

2.3 Diagnosis of Abnormally Invasive Placenta (AIP)

The abnormally invasive placenta (AIP) diagnosis was made using a set of clinical, imaging, and histopathological criteria. In this procedure, antepartum grayscale ultrasound was conducted using the Samsung HS70A diagnostic system, with the aid of color Doppler to study the placental location, vascular patterns, and potential signs of abnormal adherence. Intraoperative findings during cesarean sections aided the diagnosis. These included excessive vascularity, difficulty in placental separation, and evidence of direct placental invasion into the myometrium. In cases undergoing cesarean hysterectomy, the definitive confirmation of AIP was achieved through histopathological examination of the uterine specimen.

Diagnosis followed the guidelines of the European Working Group on Abnormally Invasive Placenta (EW-AIP) and the FIGO classification of *placenta accreta* spectrum (PAS) disorders. The severity of AIP was graded as:

- Grade I (Accreta)
- Grade II (Increta)
- Grade III (Percreta)

2.4 Assessment of Obstetric Outcomes

Maternal complications encountered in the series were cesarean hysterectomy and massive transfusion, i.e., the administration of four or more units of packed red cells within a period of two hours or the anticipation of replacement for 50% of total blood volume due to active hemorrhage. The neonatal concerns noted included preterm birth (i.e., birth before 37 weeks of gestation), low birth weight (< 2500 g), low Apgar scores (< 7) at one and five minutes of time post-birth, and meconium-stained fluid, a possible marker of fetal distress.

2.5 Statistical analysis

The data were entered and statistically analyzed using the program IBM SPSS Statistics for Windows, version 26.0. Continuous variables were expressed as means±SDs or medians (IQRs) and analyzed by ANOVA or Kruskal-Wallis test. Receiver operating characteristic (ROC) curve analysis was constructed to derive the best cut-off values of TSH and TgAb to predict AIP. A statistically significant difference was considered when p-value < 0.05.

3. Results

3.1 Comparison of demographic among AIP, PPT, and control group

Table 1 presents the demographic and clinical characteristics among three groups the Abnormally Invasive Placenta (AIP) group, the Placenta Previa without Invasion (PPT) group, and the Control group. The AIP group had the highest mean age and body mass index (BMI) (34.36±3.1 years), compared to the PPT group (31.34±3.72 years) and the Control group (25.65±3.71 years). A significantly greater proportion of women in the AIP group reported previous cesarean sections (80%) than in the PPT (54.3%) and Control (31.4%) groups. Previous curettage history was also slightly more frequent in the AIP group (14.3%) than in the PPT and Control groups (11.4% each). Gestational age at sampling and delivery showed minimal variation across groups, though birth weight was lower in the AIP group (2901.45±480.6 g). Importantly, only the AIP group exhibited abnormal FIGO classifications (grades 1, 2, and 3a), the distinctive pathological severity of AIP.

Table 1: Comparison of demographic among AIP, PPT, and control group

Demographic & Clinical Factors	AIP (N=35)	PPT (N=35)	Control (N=35)
Age (years), BMI (kg/m²)	34.36±3.1	31.34±3.72	25.65±3.71
Previous curettages (Yes)	7 (14.3%)	6 (11.4%)	4 (11.4%)
Gestational age (Sampling / Birth)	29.53±1.10 / 37.69±1.60	30.46±1.20 / 37.37±1.10	30.17±1.10 / 38.17±1.00
Previous C/S (Yes)	28 (80%)	19 (54.3%)	11 (31.4%)
Birth weight (grams)	2901.45±480.6	3125.67±425.8	3165.23±410.7
FIGO Classification (Grade 1/2/3a/Normal)	11 / 8 / 11 / 0	0 / 0 / 0 / 35	0 / 0 / 0 / 35

3.2 Comparison of thyroid-related hormonal and antibody parameters among the three study groups

Table 2 presents the comparison of thyroid-related hormonal and antibody parameters among the three study groups: Abnormally Invasive Placenta (AIP), Postpartum Thyroiditis (PPT), and healthy Control (each with N=35). Significant differences were observed in TSH (P=0.001), T_3 (P=0.030), and T_4 (P=0.005) levels among the groups. Additionally, TPOAb levels varied markedly (p<0.001), with notably lower levels in the PPT group. At birth, significant differences were also recorded in T_3 and T_4 levels (P=0.002 and P=0.001, respectively). However, TgAb levels (P=0.210) and TSH at birth (P=0.070) did not differ significantly among the groups.

Table 2: Comparison of thyroid-related hormonal among the three study groups

Parameter	AIP (N=35)	PPT (N=35)	Control (N=35)	P-Value
TSH (mIU/L)	1.49±0.62	3.55±1.35	2.12±1.78	0.001
T_3 (ng/dL)	0.162±0.010	0.157±0.010	0.171±0.010	0.030
T_4 (ng/dL)	0.137±0.010	0.121±0.010	0.145±0.010	0.005
TgAb (IU/mL)	3.59±1.93	4.72±2.86	4.53±2.69	0.210
TPOAb (ng/dL)	0.135±0.005	0.033±0.002	0.090±0.003	<0.001
TSH at birth	1.30±0.15	1.45±0.12	1.42±0.11	0.070
T_3 at birth	3.10±0.20	2.80±0.18	3.15±0.22	0.002
T_4 at birth	1.10±0.07	0.95±0.06	1.12±0.06	0.001

3.3 Cutoff value, specificity, sensitivity, and AUC of TSH and TgAb levels at early third trimester in AIP cases

Table 4.3 and the accompanying ROC curve jointly evaluate the diagnostic performance of TSH and TgAb levels in predicting abnormally invasive placenta (AIP) during the early third trimester. The table highlights that TSH, with a cutoff value of 2.25 mIU/L, achieved a sensitivity of 74.29% and specificity of 70.00%, along with a high AUC of 0.831 (95% CI: 0.77-0.89). Similarly, TgAb, with a cutoff of 2.80 IU/mL, displayed a sensitivity of 68.57% and specificity of 65.71%, with an AUC of 0.748 (95% CI: 0.68-0.83). Both markers were statistically significant (p<0.001), supporting their potential role in AIP screening.

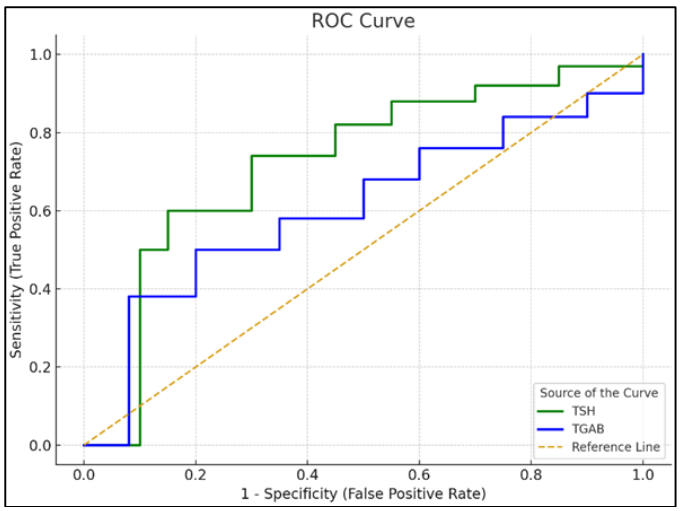

Fig 1: TSH and TgAb ROC curve in AIP

Table 3: Cutoff value, specificity, sensitivity, and AUC of TSH and TgAb levels at early third trimester in AIP cases

Metric	TSH (mIU/L)	TgAb (IU/mL)
Cut-off	2.25	2.80
Specificity	70.00%	65.71%
Sensitivity	74.29%	68.57%
AUC (95% CI)	0.831 (0.77-0.89)	0.748 (0.68-0.83)
P-Value	< 0.001	< 0.001

3.4 Logistic Regression for AIP Prediction

Table 4 presents the results of a multivariate logistic regression analysis evaluating the independent predictive value of several variables for abnormally invasive placenta (AIP). TSH emerged as a significant predictor, with an odds ratio (OR) of 0.36 (95% CI: 0.15-0.82, P=0.03), indicating that higher TSH levels are associated with a reduced likelihood of AIP. TgAb showed a borderline association (OR=0.68, P=0.04), but the confidence interval includes 1, suggesting a less robust predictive value. Interestingly, T_4 had an OR of 3.3 (95% CI: 2.1-4.8), implying a strong positive association with AIP risk, although its p-value of 0.07 did not reach conventional statistical significance. Prior cesarean section was significantly associated with reduced AIP odds (OR=0.12, P=0.008).

Table 4: Logistic Regression for AIP Prediction

Model	TSH (mIU/L)	TgAbs (IU/mL)	T_4 (ng/dL)	Previous Cesarean Section
Multivariate OR (95% CI)	0.36 (0.15-0.82)	0.68 (0.38-1.24)	3.3 (2.1-4.8)	0.12 (0.01-0.45)
P-Value	.03	.04	.07	.008

3.5 Logistic Regression for AIP Prediction

Table 5 explores the association between thyroid-related biomarkers and adverse maternal outcomes in patients with abnormally invasive placenta (AIP). A lower TSH level was significantly associated with reduced risk of cesarean hysterectomy (OR=0.24, P=0.020), suggesting a protective effect. TgAb levels also showed a borderline association with this outcome (OR=0.72, P=0.041), indicating a marginally significant reduction in risk. For massive blood transfusion, TSH did not show a significant association (P=0.130), whereas TgAb levels were significantly associated with reduced transfusion risk (OR=0.34, P=0.018).

Table 5: Association of TSH and TgAbs levels with maternal outcomes in AIP cases

Outcome	Marker	OR (95% CI)	P-Value	Significance
Cesarean Hysterectomy	TSH (mIU/L)	0.24 (0.05-0.68)	0.020	Significant ↓ risk
	TgAbs (IU/mL)	0.72 (0.39-1.15)	0.041	Marginally sig.
Massive Transfusion	TSH (mIU/L)	0.65 (0.30-1.18)	0.130	Not significant
	TgAbs (IU/mL)	0.34 (0.12-0.76)	0.018	Significant ↓ risk

3.6. Maternal outcomes by TSH and TgAb levels in AIP cases

Patients with high TSH levels (≥ 2.25 mIU/L) showed higher frequencies of cesarean hysterectomy (57.14%), massive transfusion (45.71%), ICU admission (40.00%), and *placenta accreta* (51.43%) compared to those with low TSH levels. Similarly, patients with low TgAb levels (<2.80 IU/mL) exhibited greater rates of adverse outcomes, including cesarean hysterectomy (51.43%), massive transfusion (62.86%), ICU admission (34.29%), and *placenta accreta* (57.14%), compared to those with elevated TgAb levels.

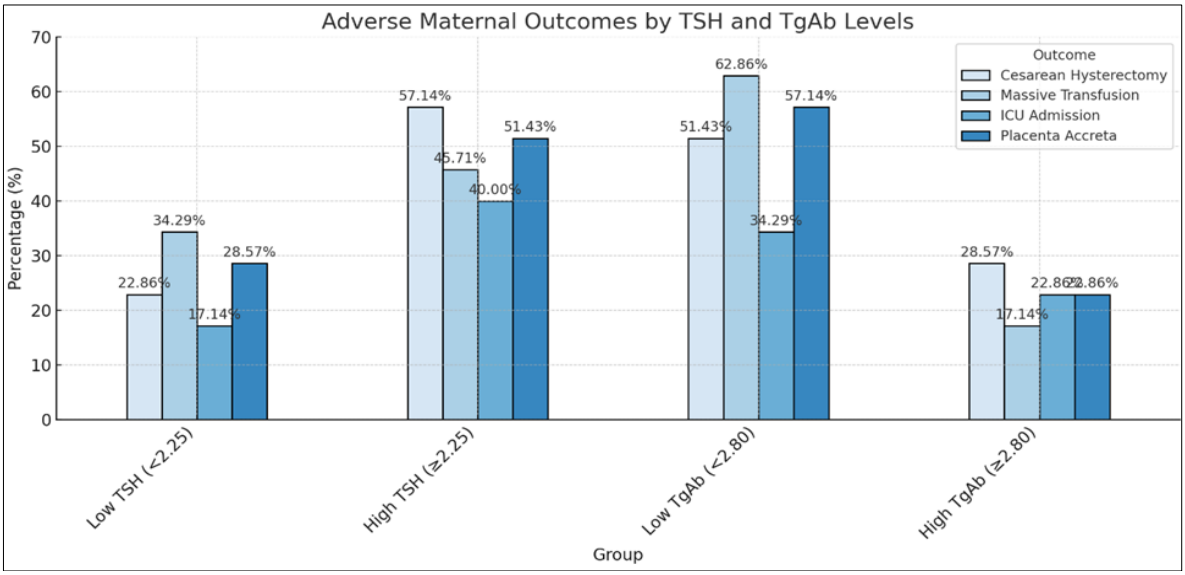

Fig 2: Maternal outcomes by TSH and TgAb levels in AIP cases

Table 6: Maternal outcomes by TSH and TgAb levels in AIP cases

Group	Cesarean Hysterectomy	Massive Transfusion	ICU Admission	*Placenta accreta*
Low TSH (<2.25)	4 (22.86%)	6 (34.29%)	3 (17.14%)	5 (28.57%)
High TSH (≥2.25)	10 (57.14%)	8 (45.71%)	7 (40.00%)	9 (51.43%)
Low TgAb (<2.80)	9 (51.43%)	11 (62.86%)	6 (34.29%)	10 (57.14%)
High TgAb (≥2.80)	5 (28.57%)	3 (17.14%)	4 (22.86%)	4 (22.86%)

3.7 Neonatal outcomes by TSH and TgAb Levels in AIP cases

Table 6 summarizes the neonatal outcomes in AIP (Abnormally Invasive Placenta) cases based on maternal TSH and TgAb levels. Neonates born to mothers with high TSH levels (≥ 2.25 mIU/L) experienced higher rates of preterm birth (62.86%), low birth weight (57.14%), NICU admission (51.43%), and low Apgar scores at 5 minutes (34.29%) compared to those with low TSH levels. Regarding TgAb levels, neonatal complications were somewhat comparable between low and high TgAb groups, though NICU admission and low Apgar scores were slightly more frequent among those with higher TgAb levels.

Table 7: Neonatal outcomes by TSH and TgAb Levels in AIP cases

Group	Preterm Birth	Low Birth Weight	NICU Admission	Apgar <7 at 5 min
Low TSH (<2.25)	8 (45.71%)	7 (40.00%)	5 (28.57%)	3 (17.14%)
High TSH (≥2.25)	11 (62.86%)	10 (57.14%)	9 (51.43%)	6 (34.29%)
Low TgAb (<2.80)	10 (57.14%)	9 (51.43%)	6 (34.29%)	4 (22.86%)
High TgAb (≥2.80)	9 (51.43%)	8 (45.71%)	8 (45.71%)	5 (28.57%)

4. Discussion

Maternal thyroid function, especially serum thyroid-stimulating hormone (TSH) and thyroglobulin antibody (TgAb), may play a role in the matrix of etiologies and clinical outcomes of abnormally invasive placenta (AIP), according to this article. It was observed that low TSH and TgAb levels in the early third trimester had a statistically significant negative association with AIP, which corroborates the observations made by Ozler *et al*. (2021) [13], showing that low levels of both markers were independently predictive of unfavorable maternal outcomes such as cesarean hysterectomy and massive blood transfusion (1).

The previously observed correlation between AIP and prior cesarean delivery in our study (80% in the AIP group) seems well grounded theoretically and empirically in the literature. Several studies have documented uterine scars from previous cesarean sections to be a predisposing factor for abnormal implantation and invasion by the placenta (2-5). Jauniaux *et al*. (2018) [14] emphasized cesarean section as the most important modifiable risk factor for the development of AIP (3), while Morlando *et al*. (2013) and Bowman *et al*. (2014) [15, 16] showed that increasing cesarean rates correlate with rising incidence of AIP (4,30).

From the endocrine point of view, our findings lend credence to an increasing body of evidence relating thyroid dysfunctions to placental pathologies. TSH and thyroid hormones are strongly implicated in trophoblast proliferation, secretion of cytokines, and angiogenesis-systems that are basic to normal placentation [17-19]. Vasilopoulou *et al*. (2014) [20] showed that T_3 modulates angiogenic cytokines in the decidua according to gestational age (11), while Rahnama *et al*. (2021) [21] have shown that the placental tissue expresses thyroid peroxidase, thereby suggesting a possibility of it being an autoimmune target in pregnancy (77).

In our ROC analysis, a cut-off value of 2.25 mIU/L had a TSH with an AUC of 0.831, so close to the report of Ozler *et al*. (2021) [22] of an AUC of 0.844 for TSH at a similar cut-off (1), indicating a strong discriminatory ability of TSH to predict AIP. TgAb showed a moderate ability for prediction (AUC=0.748) consistent with literature data [23].

In fact, low TSH and TgAb levels were correlated with and maternal morbidity factors that included increased rate of cesarean hysterectomy and massive transfusion, a discovery also described by Jauniaux *et al*. (2019) and Fitzpatrick *et al*. (2014) [24, 25], who accentuated early diagnosis to curb complications of disorders of PAS. The association of low thyroid marker levels with invasive placentation and hemorrhagic outcomes may relate to the altered expression of angiogenic markers like VEGF and sFlt-1, which are known to be dysregulated during AIP.

Most importantly, our results strongly support thyroid screening in the early third trimester as a complementary method for AIP risk stratification. While ultrasound remains the most common method for diagnosing AIP, the sensitivity of this technique is subject to variability among operators and has shown interobserver differences [13, 19, 42]. Markers such as TSH and TgAb would be ideal to be integrated with imaging modalities for better predictive accuracy, as was highlighted by Collins *et al*. (2016) and D'Antonio *et al*. (2013) [12, 13].

5. Conclusions

- The AIP group exhibited distinct demographic and clinical features, including significantly higher maternal age, BMI, and a history of cesarean sections compared to the PPT and control groups. Additionally, all abnormal FIGO classifications (grades 1, 2, and 3a) were exclusive to the AIP group, underlining its clinical severity.
- The AIP group demonstrated lower TSH and T_4 levels, while the PPT group showed markedly lower TPOAb levels. At birth, thyroid hormone levels (T_3 and T_4) also varied significantly, particularly in AIP cases, indicating a potential endocrine disruption in these pregnancies.
- ROC curve analysis revealed that both TSH and TgAb levels in the early third trimester had acceptable predictive power for diagnosing AIP, with TSH showing higher sensitivity and specificity (AUC=0.831) than TgAb (AUC=0.748).
- Logistic regression identified lower TSH as significantly predictive of reduced AIP risk. T_4 levels,

although showing a strong odds ratio, did not reach statistical significance. Previous cesarean section history remained a strong independent predictor.

- Maternal outcomes, including cesarean hysterectomy, ICU admission, and massive transfusion, were more frequent in AIP cases with high TSH and low TgAb levels, suggesting these markers may reflect increased disease severity.
- Neonatal outcomes, such as preterm birth, low birth weight, NICU admission, and low Apgar scores, were worse among newborns of mothers with high TSH levels.

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