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ORIGINAL ARTICLE

Third-Generation Thyrotropin Receptor Antibody (TRAb) assay for predicting neonatal thyroid dysfunction in pregnant women with Graves' disease

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Abstract

Purpose The aim is to validate the third generation Thyrotropin receptor antibody (TRAb) assay for predicting neonatal thyroid dysfunction and adverse pregnancy outcomes in pregnant women with Graves' disease.

Methods This prospective cohort study was conducted in TRAb positive pregnant women with Graves' disease and their off springs. The primary outcome was to assess different forms of neonatal thyroid dysfunction in relation to maternal and neonatal TRAb levels. The secondary outcome was to predict adverse pregnancy outcomes by using maternal TRAb levels. Serum T3, FT4, TSH, TRAb levels were measured using electrochemiluminescence immunoassay.

Results 51 pregnant women were included. Five women had adverse pregnancy outcomes, TRAb levels of > 19.06 IU/L (10.9 times the upper limit of normal (ULN)) predicted adverse pregnancy outcomes with 100% sensitivity and 93.5% specificity. Among the 46 successful live births, 13 (28.3%) had neonatal thyroid dysfunction. Out of 13 neonates, 7 (32%) had neonatal thyrotoxicosis, 4 (18%) had primary hypothyroidism, and 2 (9%) had central hypothyroidism. Third trimester maternal TRAb levels of > 7.99 IU/L (4.6 times the ULN)and day three neonatal TRAb levels of > 5.03 IU/L (2.9 times the ULN), predicted the neonatal thyrotoxicosis with 100% sensitivity and 97.4% specificity.

Conclusion Very high maternal third generation TRAb levels strongly predicted the adverse pregnancy outcomes and neonatal thyroid dysfunction in pregnant women with Graves' disease. Neonatal thyroid function test along with the TRAb levels strongly correlated with different forms of neonatal thyroid dysfunction and is very useful in avoiding inadvertent treatment to neonates.

Keywords Graves' disease · Thyrotropin Receptor Antibody (TRAb) · Thyrotoxicosis · Hyperthyroidism · Thyroid storm

Introduction

Graves' disease is uncommon in pregnancy and affects 0.2% of pregnant women [1]. Hyperthyroidism in pregnancy may lead to various maternal complications like pregnancy loss, preterm delivery, pre-eclampsia, thyrotoxic cardiomyopathy, and thyroid storm [2]. Fetal and neonatal complications of maternal hyperthyroidism include low birth weight, neonatal thyrotoxicosis, primary as well as central hypothyroidism. The neonatal thyroid dysfunctions are due to very high maternal thyroid hormone levels,transplacental passage of Thyrotropin receptor antibody (TRAb) as well as maternal anti-thyroid drugs. A high level of stimulating TRAb is associated with fetal/neonatal thyrotoxicosis and hypothyroidism with blocking TRAb level.

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The previous published studies were used second-generationTRAb assays in predicting the adverse pregnancy outcomes and neonatal thyroid dysfunction and few studies used first-generation assay as well [3–9]. The recent third-generation assay uses labelled monoclonal human TSHR-stimulating antibody (M22). The third-generation assay has increased sensitivity as compared to first- and second-generation assays because M22 and patients' TRAb bind to similar TSHR epitopes [10, 11]. To the best of our knowledge, this is the first study to evaluate TRAb levels by using third generation assay for predicting adverse pregnancy outcomes and neonatal thyroid dysfunction in Graves' disease mother who had not underwent any radioiodine ablation or surgical management.

Material and methods

This is a prospective cohort study conducted in a 3100 bedded multi-specialty tertiary care hospital with an established Comprehensive Emergency Obstetric and Newborn care (CEmONC) Centre, under Government of Tamil Nādu, National Health Mission, performing approximately 1000 deliveries per month, in Southern India. The study period was between January 2021 and May 2023. Inclusion criteria were all pregnant women with Graves' disease diagnosed either before or during pregnancy, with positive TRAb levels. Graves' disease was diagnosed based on symptoms and signs of hyperthyroidism, including diffuse goiter and/or Graves' ophthalmopathy with or without dermopathy along with suppressed pregnancy trimester-specific thyroid stimulating hormone (TSH), high total thyroxine (TT4) or free thyroxine (FT4) and positive TRAb levels [12]. Exclusion criteria were hyperthyroidism other than Graves' disease as well as not consenting for study. All the pregnant women who were on propylthiouracil during first trimester were converted into equivalent doses of carbimazole in the second and third trimester on aiming to keep FT4 at high normal levels. The study was approved by the Institutional Ethics Committee, Madurai Medical college and Govt. Rajaji Hospital, Madurai (CDSCO: Reg. No. ECR/13651Inst/TN2020 & DHR Reg.No.EC/NEW/INST/2020/484).

The primary outcome measures were different forms of neonatal thyroid dysfunction in relation to maternal and neonatal TRAb levels. The secondary outcome measures were to find out the maternal TRAb levels in predicting adverse pregnancy outcomes and neonatal transmission.The detailed maternal histories included were the duration of Graves' disease, antithyroid drug details and adverse effects. The various clinical parameters like APGAR score, presence of goiter, dry skin, periorbital edema, icterus, umbilical hernia,muscle tone, heart rate and rhythm were examined in neonates. Thyroid function test (TFT) that included serum FT4, total triiodothyronine (TT3), TSH were measured using electrochemiluminescence immunoassay (ECLIA) by Roche Cobas e411 analyzer, Roche diagnostics, Germany. TRAb levels were quantified with third generation TSH binding inhibiting immunoglobulins (TBII) ECLIA using Roche Cobas e411 immuno-analyzer. The assay result was considered positive when a cut off value of 1.75 IU/l and above. The measuring range, given by manufacturer, was between 0.8 and 40 IU/L with a coefficient of variation $< 6\%$, sensitivity of 97% and a specificity of 99%.

In all pregnant women, TSH and FT4 levels were monitored at baseline and once in six weeks throughout the gestation and TRAb levels were measured either at first trimester or first antenatal visit and repeated again at third trimester. Thyroid storm was diagnosed if Burch-Wartofsky Point Scale of more than 45 [2]. In all neonates TSH, FT4, TT3 was measured on day 3 as well as on day 30. After that, depend upon the clinical indications TFT were monitored once in 6 weeks to till it become normal. In term neonates, normal day 3 thyroid function test were defined as FT4- 0.86–2.49 ng/ml and TSH- 0.70–20 µIU/l. (between 2.5th and 97.5th percentiles). Overt Hypothyroidism was defined as FT4 below 0.86 ng/ml and TSH above 20 µIU /l. Overt Hyperthyroidism was defined as FT4 above 2.49 ng/ml and TSH below 0.70 µIU/l. TRAb levels were measured at day 3 and if positive measured once in 6 weeks until it became negative. All the neonates with thyroid dysfunction were treated at neonatal intensive care unit (NICU) under the care of neonatologist.

Statistcal analysis

Data were analyzed using SPS22 software. The data were described as median and interquartile range with 95% CI because of skewed distribution. The groups were compared using Mann Whitney U test for nominal data and chi-squared test for categorical data. Multiple regression analysis was performed to identify predictors of neonatal thyroid dysfunction and adverse pregnancy outcomes. Receiver operator characteristic (ROC) analysis was performed to evaluate cut off levels, sensitivity and specificity of maternal and neonatal TRAb in predicting maternal and neonatal outcomes and the Youden index was calculated. Pearson correlation coefficient test was performed to assess correlations between variables. The values of $p < 0.05$ were considered statistically significant.

Results

51 pregnant women with TRAb positive Graves' disease were included in the study. 32 (63%) were diagnosed as

Table 1 Baseline characteristics of the present study cohort.

	Pre-pregnancy onset Graves' disease ($n = 32$) Median (range)	Pregnancy onset Graves' disease ($n = 19$) Median (range)	p-value
Age at diagnosis (years)	25 (15–28)	23 (20–26)	0.317
Duration of hyperthyroidism (months)	60 (3–192) Not Applicable	7 (1–9)	< 0.001
Gestational age of Graves' disease onset (months)		3 (1–9)	
Number of thyroid storms	4	3	0.371
Number of adverse pregnancy outcomes	2	3	0.134
Number of preterm deliveries	2 (Total live birth–30)	3 (Total live birth–16)	0.108
Number of lower segment cesarean sections	16 (Total live birth–30)	5 (Total live birth–16)	0.079
Median Birth weight 0 f neonates (Kg)	2.7 (2–3.3)	2.7 (1.5–3.5)	-
Number of neonates born with thyroid dysfunction	8	5	0.489
FT4 levels at second trimester (ng/dl)	1.69 (1.00–3.91)	4.20 (1.56–7.77)	0. 011
FT4 levels at third trimester (ng/dl)	1.57 (1.00–3.00)	1.68 (1.45-4.00)	0.117
TRAb levels at first trimester (IU/L)	8.03 (2.12–35.19)	9.91 (3.18–45.41)	0.500
TRAb Levels at third trimester (IU/L)	4.96 (1.75–36.47)	3.57 (2.41–40)	0.984
Total daily dose of carbimazole during second trimester (mg)	15 (5–30)	25 (20–40)	0.189
Total daily dose carbimazole during third trimester (mg)	5 (0–30)	10 (0–40)	0.103

Graves' disease before pregnancy and 19 (37%) were during pregnancy. Baseline clinical characteristics of these women including age at diagnosis, duration of Graves's disease, median FT4 and TRAb levels at second and third trimester, total daily dose of carbimazole, incidence of thyroid storm, adverse pregnancy outcomes, mode of delivery, are compared between both these groups and the p-value were summarized in Table 1. Out of 51 pregnant women, TRAb levels were available for comparison in 39 women for both first and third trimester. In 92.3% ($n = 36$) of pregnant women, there was a significant improvement in TRAb levels between first and third trimester. However, in 7.69% ($n = 3$) of women TRAb levels worsened paradoxically despite of good drug compliance and all their offspring had thyroid dysfunction.

Seven (13.7%) out of 51 women had thyroid storm, and all of them had very high TRAb levels (median- 35.49 IU/L (21.03–40.99)). Of these 5 (71.42%) had adverse pregnancy outcome in the form of first trimester abortions ($n = 3$) and still birth ($n = 2$). ROC analysis showed a TRAb levels of > 19.06 IU/L (AUC- 0.978 95% CI) (10.9 times the upper limit of normal (ULN)) was associated with adverse pregnancy outcomes with a 100% sensitivity, 93.5% specificity and Youden index of 0.935 ($p < 0.001$). (Fig. 1a).

The live birth rate in present cohort is 90.2% (46 neonates were born to 51 pregnant women). The median birth weight is 2.75 kg (1.5–3.5). Of these 46 neonates, 22 (47.82%) had positive TRAb levels. Third trimester very high maternal TRAb levels were transmitted to their offspring. ROC analysis showed that TRAb level of > 4.27 IU/L (AUC- 0.989 95% CI) (2.4 times the ULN) in third trimester was transmitted to their neonates with 100% sensitivity, 91.7% specificity and Youden index of 0.917 ($p < 0.0001$). (Fig. 1b). The various thyroid dysfunctions of these TRAb-positive neonates are described in Fig. 2.

Seven neonates were diagnosed as neonatal thyrotoxicosis in the present cohort; all of them had transient hyperthyroidism and became euthyroid by four months with normal TRAb levels. All the neonatal thyrotoxicosis had low birth weight and the median birth weight was 2.15 (2–2.3) Kg. Three out of these seven neonates were symptomatic at birth and required anti-thyroid therapy. Table 2 compared the maternal FT4 level, TRAb level, and total daily dose of carbimazole at second trimester and before delivery as well as the clinical characteristics, FT4 and TRAb levels of neonates with thyrotoxicosis. Mother with very high third trimester TRAb levels are associated with increased risk of neonatal thyrotoxicosis. ROC analysis showed that maternal TRAb levels of > 7.99 IU/L (AUC-0.978) (4.6 times the ULN) predicted neonatal thyrotoxicosis with 100% sensitivity, 97.4% specificity and Youden index of 0.974 ($p < 0.0001$) (Fig. 1C). The day 3 neonatal TRAb levels may be also used as a predictor of neonatal thyrotoxicosis. The ROC analysis showed that neonatal TRAb levels of > 5.03 IU/L (AUC-0.989 CI 95%) (2.9 times the ULN) predicted neonatal thyrotoxicosis with 100% sensitivity, 97.4% specificity and a Youden index of 0.974 ($p < 0.0001$). (Fig. 1D).

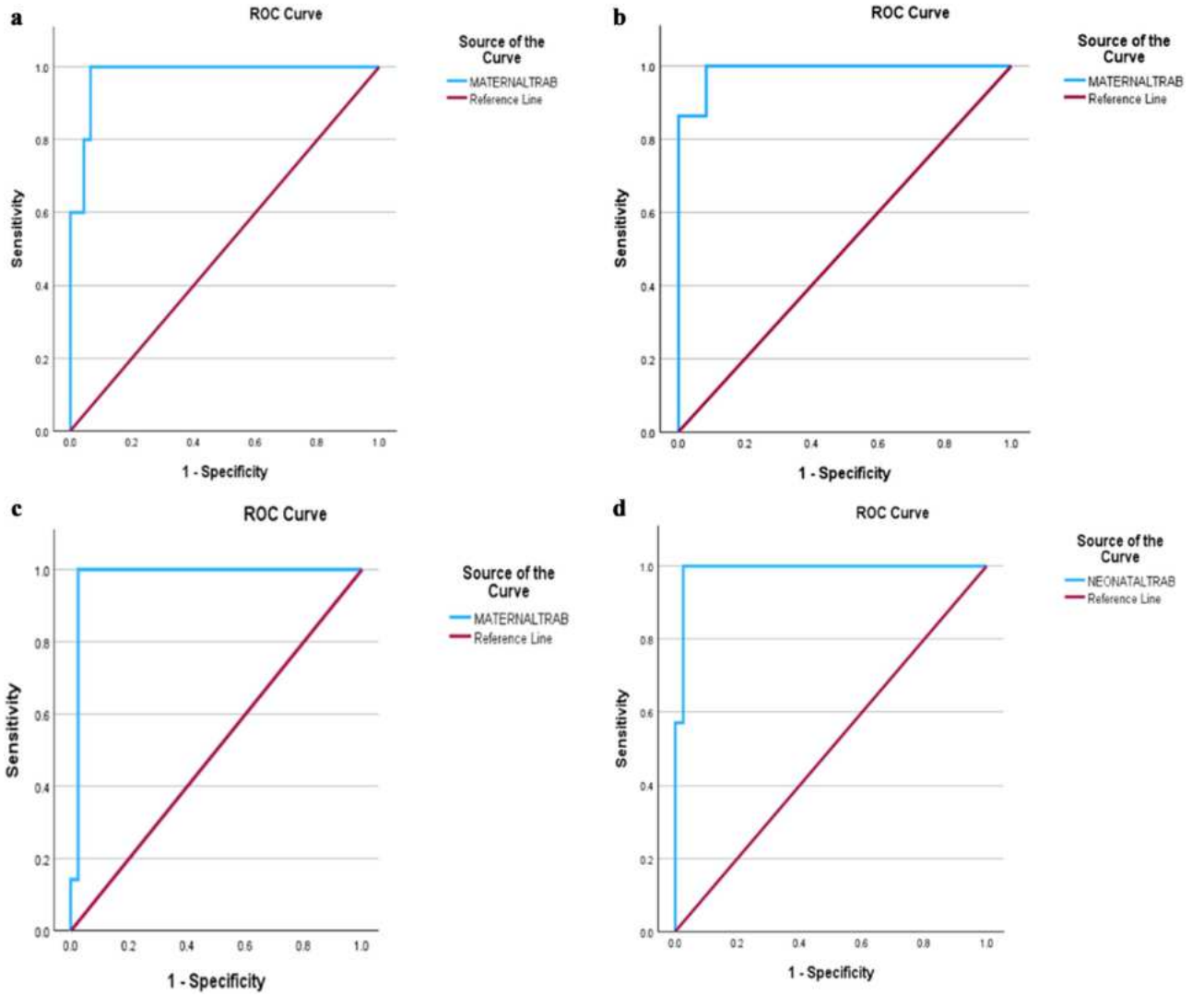

Fig. 1 **a** Maternal TRAb levels predicting adverse pregnancy outcomes. **b** Maternal TRAb levels predicting neonatal transmission. **c** Maternal TRAb levels predicting neonatal thyrotoxicosis. **d** Neonatal TRAb levels predicting transient thyrotoxicosis

Ten neonates had transient hypothyroidism, out of them six were TRAb positive and four were TRAb negative. Among the TRAb positive hypothyroid neonates, four were primary and two had central hypothyroidism. All the TRAb positive hypothyroidism was treated with six months of Levothyroxine therapy, till TRAb level became negative. The comparison of maternal FT4, TRAb level and total daily dose of carbimazole at second trimester and before delivery as well as clinical characteristics, FT4 and TRAb levels of neonates with hypothyroidism are described in Table 3. Four TRAb negative neonatal hypothyroidism were improved spontaneously without any treatment; the possible mechanisms are due to maternal antithyroid drug related transient hypothyroidism. Neonatal TRAb levels are very well correlated with maternal TRAb levels (r (46) = 0.453, $p = 0.002$) (Fig. 3) in the present study. Only one neonate had (2.17%) anti-thyroid drug related adverse effect, bilateral cleft lip and palate due to high maternal carbimazole dose in first trimester. (Supplementary Fig. 1). The various TRAb cutoffs that predicted the adverse pregnancy outcomes and neonatal thyroid dysfunctions are summarized in Table 4. The multiple linear regression analysis revealed that the maternal TRAb is the strongest independent predictor of adverse pregnancy outcomes (Table 5a) and neonatal thyrotoxicosis (Table 5b).

Discussion

The Graves' disease complicating pregnancy is a challenging situation to manage and poorly controlled hyperthyroidism is associated with adverse pregnancy outcomes and various neonatal complications. There are very limited studies in the published literature regarding the usefulness of TRAb levels

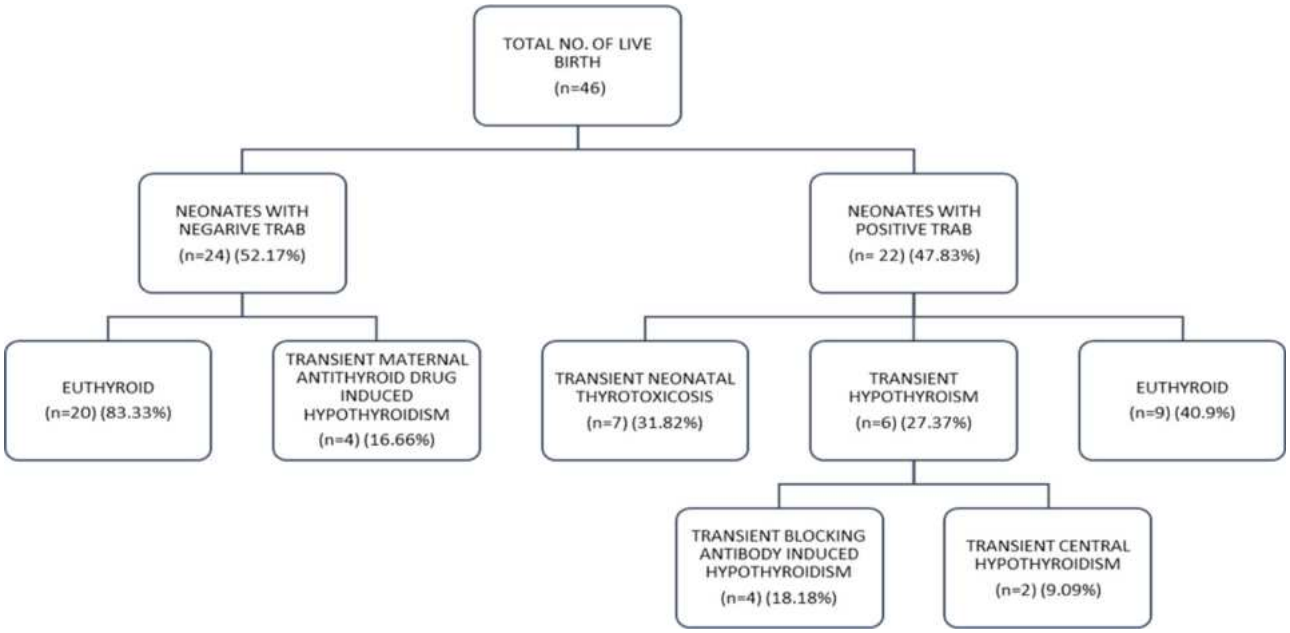

Fig. 2 Clinical characteristics of neonates with positive TRAb levels

in predicting clinical outcomes in both pregnant women as well as in newborn. In addition, most of the previous studies were retrospective and used either first- or second-generation TRAb assays [1, 3–9]. To the best of our knowledge, this is the first prospective study using third generation TRAb assay in predicting maternal, fetal and neonatal outcomes in Graves' disease mother who had not undergone any radio-iodine ablation or thyroid surgical procedures.

The TRAb assays are of two types, "receptor assays" and "bioassays" [13]. Receptor assays measure TSH binding inhibiting immunoglobulins (TBII) that detect serum auto-antibodies by their capacity to compete for the binding of labelled TSH to an TSH receptor preparation. Whereas bioassays detect the functional properties of TRAbs, i.e., stimulating (TSABs) or blocking (TBABs). TBII assays have undergone changes over years. The first-generation assays used porcine thyroid membrane extracts, radi-olabelled TSH in liquid phase and the second-generation assays used fluorescent labelled bovine TSH, recombinant human or porcine thyroid membrane extracts in solid phase. The third-generation assay uses inhibition of binding of labelled monoclonal human TSHR-stimulating antibody M22. The advantage of using the monoclonal anti-TSHR antibody is increased sensitivity because M22 and patients' TRAb bind to similar TSHR epitopes [10]. Overall, the sensitivity of the second- and third-generation TRAb assays are 86.5% and 97%, and specificity are 97.4% and 99.2%, respectively [14].

In the present study, both the pre pregnancy as well as pregnancy onset Graves' disease mother was included. In spite of median age at presentation of pregnancy onset Graves' disease is 7 months, there were no statistically significant differences in FT4 as well as TRAb levels at third trimester /at the time of delivery. Maternal TRAb levels and antithyroid drug requirement decreased in third trimester in most of our patients, however paradoxically increased in 8% patients. This is similar to study by Abeillon-du Payrat et al. [3], where TRAb levels decreased or remained stable in 86% of patients by third trimester and increased in 14%. This decrease in antibody levels in third trimester are due to antithyroid drug treatment related well controlled Graves' disease as well as physiological immunosuppression in pregnancy [15]. Nevertheless, Graves' disease's course is highly variable in pregnancy, and there may be a rise in TRAb levels in severe and/or newly diagnosed Graves' disease as well as co-existing other obstetrical co-morbidities like diabetes mellitus, hypertension, preeclampsia and renal dysfunction that may worsen or unmask the underlying Graves' disease [16]. Hence, TRAb levels should be done in all pregnant women with Graves' disease since it the strongest predictor of adverse obstetric outcome as well as neonatal thyroid dysfunction [6].

In the present study, seven women had thyroid storm, out of which five had adverse pregnancy outcome in the form of first trimester miscarriage and still birth. This is similar to the other studies reported in literature [15, 16]. In addition to hyperthyroidism, other co-morbidities like preeclampsia, diabetes, hypertension might also cause adverse pregnancy and neonatal outcomes [17]. However, in the present study, none of the Graves' disease mother had these co-morbidities. TRAb levels of more than 19.06 IU/L (10.9 times ULN) were associated with adverse pregnancy

Table 2 Comparison of maternal and neonatal clinical characteristics, thyroid hormone and TRAb levels in neonatal thyrotoxicosis

Maternal						Neonatal						
Case	FT4 level at second trimester(ng/dl)	FT4 level before delivery (ng/dl)	Third trimester TRAb levels(IU/L)	CBZ dose at second trimester(mg)	CBZ dose before delivery(mg)	Clinical manifestations	Birth weight(Kgs)	Day 3 TRAb levels (IU/L)	FT4 levels at presentation (ng/ml)	TSH levels at day 3 (μIU/ml)	Treatment	Time to became TRAb-ve
1.	1.75	1.48	36.47	25	20	Tachycardia, Respiratory distress	2	10.97	3.95	0.01	CBZ[a] 1 mg/kg for 3 months	3 months
2.	1.56	1.45	8.68	20	15	None	2.3	4.49	2.55	0.08	-	3 months
3.	1.65	1.68	9.49	10	10	None	2.2	5.68	2.64	0.04	-	3 months
4.	1.35	1.52	17.51	20	20	Respiratory distress, Volume overload at day 7.	2	6.44	3.57	0.05	CBZ[a] 1 mg/kg for 4 months	4 months
5.	1.70	1.66	14.02	20	20	None	2.4	6.54	2.69	0.05	-	4 months
6[a].	5.5	1.65	16.43	30	25	Tachycardia at day 7.	2.15	5.55	3.77	0.06	CBZ[a] 1 mg/kg for 3 months	3 months
7[a].	7.77	1.70	21.03	30	25	None	2.15	5.68	2.72	0.04	-	4 months

[a]Case 6,7 are pregnancy onset Graves' disease, whereas remaining all are pre pregnancy onset.

Table 3 Comparison of maternal and neonatal clinical characteristics, thyroid hormone and TRAb levels in neonatal hypothyroidism

Maternal					Neonatal						
Case	FT4 level at second trimester(ng/dl)	FT4 level at before delivery(ng/dl)	CBZ dose at second trimester(mg)	CBZ dose before delivery(mg)	Clinical diagnosis	Day 3 TRAb levels (IU/L)	FT4 levels(ng/dl)	TSH levels(μIU/ml)	Treatment	Time to became TRAb-ve	Time to Normal FT4
1.	1.45	1.56	10	10	Primary hypothyroidism	2.5	0.80	28.88	Thyroxine 10 μg/kg	3 months	2 months
2.	1.65	1.4	30	25	Primary hypothyroidism	4.51	0.64	35.47	Thyroxine 10 μg/kg	4 months	3 months
3.	1.56	1.67	10	10	Primary hypothyroidism	3.43	0.59	50.32	Thyroxine 10 μg/kg	6 months	2 months
4.	3.50	1.72	30	40	Central hypothyroidism	2.36	0.79	0.66	Thyroxine 10 μg/kg	4 months	2 months
5[a].	2.85	1.63	20	15	Primary hypothyroidism	5.74	0.93	25.89	Thyroxine 10 μg/kg	4 months	2 months
6[a].	7.77	1.90	30	40	Central Hypothyroidism	2.74	0.85	0.75	Thyroxine 10 μg/kg	5 months	1 month

[a]Case 5, 6 are pregnancy onset Graves' disease,whereas remaining all are pre pregnancy onset.

outcome. The above third generation TRAb level has a high sensitivity (100%) and specificity (93.5%) as compared to previous study that used second generation TRAb with a sensitivity and specificity of 83.5% and 85.3%, respectively with the cutoff of 3.5 IU/L [17]. Thus, very high TRAb levels are a sensitive indicator to assess the adverse pregnancy and fetal outcomes. Not only the maternal TRAb levels, but also maternal thyroid function as well as the effects of anti-thyroid drugs play an important role in predicting adverse pregnancy outcomes and neonatal thyroid dysfunction. However the multiple linear regression analysis revealed that the maternal TRAb is the strongest independent predictor of adverse pregnancy outcomes and neonatal thyroid dysfunction.

Due to diligent clinical and biochemical follow-up, most of the study population were euthyroid in the third trimester irrespective of TRAb levels. Among the 46 successful live births, 22 women transmitted the TRAb to their off springs. ROC analysis showed that third generation maternal TRAb levels of more than 4.27 IU/L was transmitted to their neonates with a sensitivity of 100% and specificity of 91.7%. The previous studies had not demonstrated any specific TRAb cut off at which there was a risk of neonatal transmission [1, 3]. Hence the present study highlighted that mere maternal TRAb positivity alone is not associated with neonatal thyroid dysfunction. The third trimester maternal TRAb level of more than 2.4 times ULN alone was transmitted to their off springs, and the transmission occurs irrespective of maternal thyroid function status.

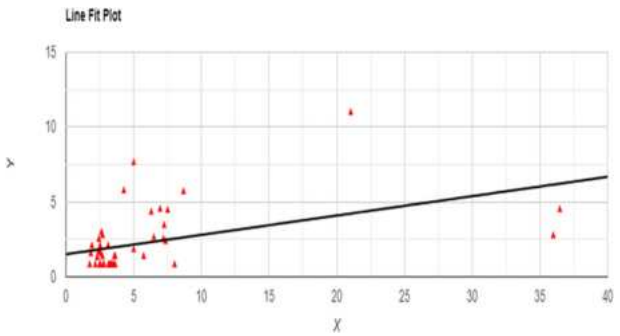

Fig. 3 Correlation between maternal and neonatal TRAb levels

Among the 22 TRAb-positive neonates, seven had thyrotoxicosis. One was symptomatic at birth, two were symptomatic by Day 7, in the form of respiratory distress, tachycardia, and were treated with carbimazole 1 mg/kg. Four were asymptomatic, did not require any anti-thyroid drug and all of them were closely monitored.

Due to the trans-placental transfer of maternal anti-thyroid drugs in neonatal circulation, mere normal TFT on day three alone does not exclude neonatal thyrotoxicosis. Once the effect of maternal anti-thyroid drug disappears in neonatal circulation from day 10 onwards, the neonate may become symptomatic with elevated thyroid hormone levels. An approach to the management of neonates born to mothers with Graves' disease by van der Kaay DC et al. [18] recommends that TFT in newborn should be measured on day 3 of life and if it is normal it should be repeated at day 10. Hyperthyroidism in all these neonates was transient, thyroid functions and TRAb levels were normalized in circulation by four months. This is similar to previous studies reported in published literature [3, 4]. Very high third trimester maternal TRAb levels of more than 7.99 IU/L (4.6 times the ULN) predicted neonatal thyrotoxicosis with a 100% sensitivity and 97.4% specificity. However, the present third-generation TRAb cut-off is higher than previous study by Abeillon-du Payrat et al. [3] and Banigé et al. [6] where a TRAb cut-off of 5 IU/l and 5.6 IU/L predicted the neonatal hyperthyroidism with a sensitivity of 100% and 100%, and specificity of 43% and 61% respectively. The higher cut-off in the present study is associated with better specificity with similar sensitivity. Maternal TRAb levels, maternal thyroid function and anti-thyroid drug play a important role in predicting adverse pregnancy outcomes and neonatal thyroid dysfunction. However, multiple linear regression analysis revealed that the maternal TRAb is the strongest independent predictor of neonatal thyrotoxicosis. Day three neonatal TRAb levels of more than 5.03 IU/L (2.9 times the ULN) in the present study predicted neonatal thyrotoxicosis with a sensitivity of 100% and specificity of 97.4%. The studies by Abeillon-du Payrat et al. [3] and Banigé et al. [6] showed that the cut-off value of 6 IU/L and 6.8 IU/L, predicted neonatal thyrotoxicosis, with 100% sensitivity by both studies and specificity of 53% and 94%

Table 4 The correlation between different TRAb levels cut-off associated with various adverse maternal and neonatal outcomes

	Mean TRAb levels	Upper limit of normal	Sensitivity	Specificity	Youden index	Correlation with adverse outcome parameters
Maternal third trimester TRAb						
1.	19.06 IU/L	10.9 times	100%	93.5%	0.935	Adverse pregnancy outcomes
2.	4.27 IU/L	2.4 times	100%	91.7%	0.917	Neonatal transmission
3.	7.99 IU/L	4.6 times	100%	97.4%	0.974	Neonatal thyrotoxicosis
Neonatal day 3 TRAb						
1.	5.03 IU/L	2.9 times	100%	97.4%	0.974	Neonatal thyrotoxicosis

Table 5 a: The multiple linear Regression model -risk factors for adverse pregnancy outcomes. b: The multiple linear Regression model -risk factors for neonatal thyrotoxicosis

PARAMETERS	Beta	*t*	sig
a			
Third trimester maternal TRAb	0.615	5.122	< 0.001
Median maternal FT4	0.244	1.989	0.053
Median anti thyroid drug dose	0.040	0.381	0.705
b			
Third trimester maternal TRAb	0.743	4.940	< 0.001
Median maternal FT4	0.147	0.994	0.326
Median anti thyroid drug dose	0.329	1.957	0.057

respectively. In summary the third generation TRAb assay cut-off has similar sensitivity with very high specificity as compared to the first- and second-generation assay.

Transient neonatal hypothyroidism is a rare form of neonatal thyroid dysfunction and is due to blocking nature of TRAb antibody [19]. In the present study, four cases of transient neonatal hypothyroidism were diagnosed. In spite of absence of classical signs of hypothyroidism, all these neonates were treated with levothyroxine for four months until TRAb levels became negative as per the recent guidelines [18]. This is similar to study by Benlarbi et al., where two cases of transient hypothyroidism were diagnosed [4]. In present study two of the neonates had central hypothyroidism. Central hypothyroidism is very rarely reported in previous studies [4, 20] In pregnant mother with severe hyperthyroidism, very high T4 may cross the placenta and suppress neonatal hypothalamic- pituitary -thyroid axis and lead to central hypothyroidism. All the central hypothyroidism neonates were evaluated for other pituitary hormone deficiencies like central hypocortisolism and growth hormone deficiency and none of them had it. Again, the central hypothyroidism also was transient and required treatment for only six months till the axis became normal.

Four neonates had primary hypothyroidism in spite of negative TRAb levels. Due to the negative TRAb levels and absence of signs of hypothyroidism, they were closely monitored without any treatment till they became euthyroid. This transient hypothyroidism is due to trans-placental transfer of anti-thyroid drugs that suppresses the neonatal thyroid as well as the effects of these antithyroid drugs are cleared from neonatal circulation in three to five days [3, 4]. Thus the, neonatal TRAb levels is a useful biomarker marker that will help to decide whether to initiate either levothyroxine or anti-thyroid drugs. Nine neonates were TRAb positive but biochemically euthyroid. This is similar to previous study by Benlarbi et al. [4]. However, the euthyroidism in these neonates may reflect the equal presence of both stimulating and blocking activities [4]. Hence all TRAb positive neonates won't have thyroid dysfunction and most importantly TRAb titer levels rather than mere TRAb positivity or negativity, will predict neonatal thyroid dysfunction. Among 46 successful live births, cleft lip and cleft palate was noted in only one neonate, that was similar to previous study by Li X et al. [21].

The strengths of the present study are, first prospective study in Graves' disease mother who had not undergone any radioiodine ablation, and assessed both adverse pregnancy outcomes in mother as well as thyroid dysfunction in neonates [22, 23] Also we used third generation TRAb assay whereas the previous studies used first- or second-generation assays and most of them were retrospective in nature. From the first antenatal visit, all the pregnant mothers were meticulously followed throughout the gestation till delivery as well as in the postpartum period. All the neonates were monitored by neonatologist in neonatal ICU and treated appropriately with either levothyroxine or carbimazole therapy, and followed-up till TRAb levels were becoming normal. Another important strength is none of the pregnant women had other co-morbidities that may affect maternal and neonatal outcome. All forms of neonatal thyroid dysfunction, including central hypothyroidism were reported. In addition to all the above strengths, the multiple regression analysis were used to predict the outcomes and defined that at what TRAb levels, we may anticipate the adverse obstetrics outcome as well as neonatal thyroid dysfunction. Limitations are not using biological assays that measures generation of cyclic AMP generation in human thyroid cell culture which would have given insight into nature of either stimulating or blocking TRAb antibodies.

Conclusion

Very high maternal third generation TRAb levels are strongly predicted the adverse pregnancy outcomes as well as neonatal thyroid dysfunction in pregnant women with Graves' disease. Neonatal thyroid function test along with the maternal and neonatal TRAb levels are strongly correlated with different forms of neonatal thyroid dysfunction as well as very useful in avoiding inadvertent treatment to neonates.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12020-023-03569-3.

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Author contributions R.P. was involved in Collection of data and was involved preparation of the manuscript for submission. S.S. was involved in the in the concept, design and final approval of the manuscript. N.S. was involved in workup of antenatal mother and management. A.J. was involved in management of neonates and follow up. V.N. was involved in workup of patients and management.

E.S. was involved in supervision of workup, K.R. was involved in statistical analysis.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Consent to participate Informed consent was obtained from all individual participants and written consent was obtained from parents for neonates included in the study.

Consent to publish The authors affirm that participants parents provided informed consent to publish supplementary figures.

Ethical approval The study was performed in line with the principles of Declaration of Helsinki. Approval granted by the Institutional Ethics Committee, Madurai Medical college and Govt. Rajaji Hospital, Madurai (CDSCO: Reg. No. ECR/13651Inst/TN2020 & DHR Reg.No.EC/NEW/INST/2020/484).

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