



Thyrotropin receptor antibody: A novel risk indicator for pregnancy loss

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ARTICLE INFO

Keywords:

TRAb
Thyroid function
Pregnancy loss
Adverse pregnancy outcomes

ABSTRACT

Background: Thyroid autoantibody has been associated with adverse pregnancy outcomes. However, thyroid-receptor antibody (TRAb) has not been considered as a potential risk assessment indicator for adverse pregnancy outcomes. Therefore, we assessed the role of TRAb in evaluation of the risk of adverse pregnancy outcomes.

Methods: Pregnant women residing in Chongqing were enrolled in the study from 2012 to 2014. The TRAb, thyroid-stimulating hormone (TSH), free thyroxine (FT₄), and free triiodothyronine (FT₃) of all patients were analyzed via electrochemiluminescence assays. All data were recorded and analyzed statistically using SPSS.

Results: A total of 468 pregnant women were included in the analysis. TRAb levels were higher in women with adverse pregnancy outcomes than those in women without adverse pregnancy outcomes. The incidence rate of pregnancy loss was significantly higher in the TRAb-positive group than that in the negative group, but this difference was not found in preterm delivery and early preterm delivery. In the logistic regression model, TRAb was an independent risk factor for pregnancy loss, but not for preterm delivery and early preterm delivery. The optimal cutoff point for TRAb was 3.53 IU/L, and the sensitivity and specificity of TRAb to assess the risk of pregnancy loss are 83.5% and 85.3%, respectively. Receiver-operating characteristic (ROC) curves revealed that TRAb was superior to the combination of TSH, FT₄ and FT₃ as an indicator for assessment.

Conclusions: TRAb as a more sensitive indicator providing valuable detection to assess the potential risk of pregnancy loss, and it can be used as an effective tool to improve the clinical management of thyroid disease in pregnant women.

1. Introduction

Approximately 10% of the general population suffers from the autoimmune thyroid diseases (ATD) and at least 2–3% of pregnant women are affected by thyroid dysfunction across the world [1]. It is widely known that adverse pregnancy outcomes are associated with thyroid function disturbances in pregnancy [2]. Furthermore, thyroid autoimmunity appears to be associated with pregnancy complications, including miscarriage and preterm delivery [3]. Therefore, it is important to increase awareness on preventing complications related to autoimmune thyroid diseases and their treatment during pregnancy.

Thyroid-receptor antibody is a significant autoantibody which was detected in patients with ATD [4], including thyroid-stimulating antibody (TSAb) and thyroid-stimulating blocking antibody (TSBAb). TRAb combines with the active site of the of thyroid-stimulating hormone

receptor (TSHR) to produce different biological effects [5]. Previous studies have described TRAb as one of the main reasons causing Graves' disease and it is a valuable diagnostic indicator in ATD [6]. TRAb is useful for identifying mild hyperthyroidism without goiter and Graves' eye disease with euthyroidism [7]. Additionally, the incidence of thyroid autoantibody is higher in women with recurrent fetal loss, infertility, or miscarriage than that in women of childbearing age with no history of miscarriage [8]. High levels of TRAb may affect fetal and neonatal thyroid function and induce fetal hyperthyroidism in some cases [9,10]. The risk of fetal/neonatal thyrotoxicosis increases by 3–5 fold in cases in which TRAb values are above normal [11,12]. Thus, TRAb is crucial not only for patients with recent iodide loads in whom thyroid imaging is contraindicated or unhelpful, but also to evaluate the risk of fetal hyperthyroidism in pregnant women and identify neonates with transient hypothyroidism [13–15].

Abbreviations: TRAb, Thyroid-receptor antibody; TSH, thyroid-stimulating hormone; FT₄, free thyroxine; FT₃, free triiodothyronine; TSAb, thyroid-stimulating antibody; TSBAb, thyroid-stimulating blocking antibody; TSHR, thyroid-stimulating hormone receptor; ATD, autoimmune thyroid disease; ROC, receiver-operating characteristic curve; OR, odds ratios; 95% CI, 95% confidence intervals; AUC, area under the ROC curve

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<https://doi.org/10.1016/j.clinbiochem.2018.11.017>

Received 22 August 2018; Received in revised form 22 November 2018; Accepted 29 November 2018

Available online 01 December 2018

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A valuable indicator of thyroid diseases will contribute to the development of new screening methods, monitoring thyroid function status, and estimating the risk of adverse pregnancy outcomes. In this study, we aimed to evaluate the role of TRAb in the risk assessment of adverse pregnancy outcomes.

2. Materials and methods

2.1. Participants

This study recruited pregnant women in Southwest Hospital from 2012 to 2014. Their individual information and obstetric test results within the first 12 weeks of gestation were collected. Given that the overall rate of smoking is very low among pregnant women, smoking habit was not investigated in this study. Women with thyroid-stimulating hormone (TSH) levels < 4.0 mIU/L and no Graves' disease were included. Meanwhile, those with hypothyroidism, hyperemesis gravidarum, multiple pregnancy, pre-existing thyroid disease, or other endocrine diseases in pregnancy; aged < 20 or > 45 years; or on medication that might alter thyroid function were excluded. Maternal and neonatal outcomes were followed and prospectively assessed via record reviews and interviews. The recorded adverse pregnancy outcomes were classified as follows: pregnancy loss (including miscarriage that happened within the first 20 weeks of gestation and stillbirth that occurred after 20 weeks of gestation), preterm delivery (delivery time from 34 to 37 weeks of gestation), and early preterm delivery (delivery time < 34 weeks of gestation). The participants were divided into the TRAb positive (i.e., TRAb \geq 1.75 IU/L) and negative group (i.e., TRAb < 1.75 IU/L) according to the test results.

2.2. Sample collection

Blood samples were collected in 5-mL vacutainers (Kehua Bio-engineering Co. Ltd., Shanghai, China) via venipuncture and stored at 4 °C. Serum was separated via centrifugation at 3000 \times g for 5 min and then used for testing.

2.3. TSH, FT₄, and FT₃ detection

The serum concentrations of TSH were measured using commercially available electrochemiluminescence assays from Roche Diagnostics (Roche Diagnostics GmbH, Penzberg, Germany). FT₄ and FT₃ levels were determined via Elecsys FT₄ and Elecsys FT₃ assays, respectively (Roche Diagnostics GmbH, Penzberg, Germany). TSH, FT₄, and FT₃ were measured simultaneously according to the manufacturer's instruction. The reference limit in the manufacturer's manual was verified locally. The reference interval of normal TSH, FT₄, and FT₃ is 0.27–4.2 mIU/L, 12–22 pmol/L, and 2.8–7.1 pmol/L, respectively.

2.4. TRAb assay

TRAb levels were detected via automatic electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instruction. The reference limit in the manufacturer's manual was verified locally. The analytical range of TRAb testing is 0.3–40 IU/L, and the reference interval of TRAb testing is \leq 1.75 IU/L.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS 20.0 (SPSS Co. Ltd., Chicago, USA) and significance was set at $P < .05$. Categorical variables were analyzed via independent samples *t*-test or Chi-square test. Logistic regression analysis was used to assess the influencing factors of adverse pregnancy outcomes. The independent variables included age, body mass index, goiter, previous babies, previous

Table 1

The analysis of clinical characteristics in participants.

	TRAb positive (n = 368)	TRAb negative (n = 100)	P-value
Age	29.40 \pm 5.20	28.10 \pm 4.02	0.46 ^a
Body mass index (kg/m ²)	21.40 \pm 2.20	22.10 \pm 2.50	0.41 ^a
Goiter (%)	8.42	5.00	0.26 ^b
Previous babies (%)	42.12	45.00	0.61 ^b
Previous miscarriage (%)	8.97	6.00	0.34 ^b
Previous preterm delivery (%)	4.08	6.00	0.41 ^b
Previous early preterm delivery (%)	2.17	1.00	0.65 ^b
Pregnancy loss (%)	21.74	11.00	0.02 ^b
Preterm delivery (%)	11.96	7.00	0.16 ^b
Early preterm delivery (%)	4.89	2.00	0.21 ^b

^a Independent sample *t*-test to test statistical difference in the distribution with each group.

^b Chi-square trend test was used to test statistical difference in the distribution with each group.

miscarriage, previous preterm delivery, previous early preterm delivery, and TRAb. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for all associations.

3. Results

3.1. Participant characteristics

A total of 468 pregnant women participated in this research from 2012 to 2014 (Table 1). Tests were conducted during the first trimester of pregnancy. There were more participants in the TRAb-positive group than that in the TRAb-negative group (368 vs. 100, $P < .05$). Maternal age, pregnancy history, and body mass index did not significantly differ between the two groups ($P > .05$). Additionally, there was no significant difference in the proportion of participants with goiter in the TRAb-positive and TRAb-negative groups (8.42% vs. 5.00%, $P > .05$). There was also no significant difference in the incidence rate of preterm delivery and early preterm delivery between the TRAb-positive group and TRAb-negative group (11.96% vs. 7.00% and 4.89% vs. 2.00%, $P > .05$). However, the incidence rate of pregnancy loss was significantly higher in the TRAb-positive group than that in the TRAb-negative group (21.74% vs. 11.00%, $P < .05$).

3.2. Association between TSH, FT₄, and FT₃ levels and pregnancy outcomes

Maternal thyroid function is the most practical index reflecting fetal thyroid function in clinical practice, and the status of thyroid function is closely related to pregnancy outcomes. In general, the status of thyroid function is determined according to TSH, FT₄, and FT₃ levels in serum. To define the thyroid functional status of the participants, TSH, FT₄, and FT₃ levels were assessed and compared statistically (Table 2).

Our results show that the TRAb-positive group had lower serum TSH levels than the TRAb-negative group (1.08 vs. 1.58 mIU/L, $P < .01$). The TSH level was lower in women with pregnancy loss than that in women without pregnancy loss (0.86 vs. 1.24 mIU/L, $P < .01$). However, the TSH level of women with preterm delivery was closer to that of women without preterm delivery without any significant difference (1.17 vs. 1.19 mIU/L, $P > .05$). Similar results were obtained for the difference in TSH levels between women with early preterm delivery and those without early preterm delivery (1.04 vs. 1.20 mIU/L, $P > .05$).

The FT₄ and FT₃ levels were significantly higher in the TRAb-positive women than in the TRAb-negative (41.60 vs. 15.28 pmol/L and 7.69 vs. 6.35 pmol/L, respectively, $P < .01$). The concentration of FT₄ and FT₃ was higher in women with pregnancy loss than that in women without pregnancy loss (42.68 vs. 34.36 pmol/L and 8.70 vs. 7.09 pmol/L, respectively, $P < .01$). The FT₄ levels were higher in

Table 2
The serum concentration of TSH, FT₄, FT₃ and TRAb in participants.

Group	Thyroid function test			TRAb (IU/L)
	TSH(mIU/L)	FT ₄ (pmol/L)	FT ₃ (pmol/L)	
TRAb positive	1.08 ± 0.26	41.60 ± 9.33	7.69 ± 1.74	3.22 ± 1.17
TRAb negative	1.58 ± 0.20	15.28 ± 5.72	6.35 ± 0.73	0.86 ± 0.37
<i>P</i> -value ^a	0.00	0.00	0.00	0.00
Pregnancy loss	0.86 ± 0.21	42.68 ± 10.10	8.70 ± 2.14	4.28 ± 1.10
No Pregnancy loss	1.24 ± 0.30	34.36 ± 14.16	7.09 ± 1.37	1.34 ± 0.42
<i>P</i> -value ^a	0.00	0.00	0.00	0.03
Preterm delivery	1.17 ± 0.28	38.20 ± 10.48	8.67 ± 1.67	2.77 ± 1.16
No Preterm delivery	1.19 ± 0.32	35.70 ± 14.20	7.25 ± 1.61	2.71 ± 1.46
<i>P</i> -value ^a	0.14	0.01	0.52	0.00
Early preterm delivery	1.04 ± 0.26	38.00 ± 11.68	8.53 ± 1.03	2.82 ± 1.00
No Early preterm delivery	1.20 ± 0.33	35.88 ± 13.95	7.35 ± 1.68	2.72 ± 1.44
<i>P</i> -value ^a	0.17	0.12	0.04	0.02

^a Independent sample *t*-test was used to test statistical difference in the distribution with each group.

women with preterm delivery than that in women without preterm delivery (38.20 vs. 35.70 pmol/L, $P < .01$), but FT₄ concentration was not significantly higher in women with early preterm delivery than that in women without early preterm delivery (38.00 vs. 35.88 pmol/L, $P > .05$). By contrast, the FT₃ levels were not higher in women with preterm delivery than that in women without preterm delivery (8.67 vs. 7.25 pmol/L, $P > .05$), but the FT₃ levels were higher in women with early preterm delivery than that in women without early preterm delivery (8.53 vs. 7.35 pmol/L, $P < .05$).

3.3. Association between TRAb levels and pregnancy outcomes

TRAb is a novel indicator of thyroid function status. It plays an important role in Graves' disease. Our results indicate a higher rate of adverse pregnancy outcomes in the TRAb-positive group. However, the association between TRAb levels and adverse pregnancy outcomes is currently unclear. To further clarify this association, we analyzed the difference of TRAb level in pregnant women (Table 2).

The mean TRAb concentration in the positive and negative group was 3.22 and 0.86 IU/L, respectively. Additionally, TRAb concentration was significantly higher in women with pregnancy loss than that in women without pregnancy loss ($P < .01$). The average TRAb level reached 4.28 IU/L in the pregnancy loss group, and it was 3.19-fold higher than that in women without pregnancy loss. Moreover, TRAb value was slightly higher in women with preterm delivery and early preterm delivery than that in women without preterm delivery and early preterm delivery, and the difference was statistically significant ($P < .05$).

3.4. Influencing factors of adverse outcomes in pregnant women

Adverse pregnancy outcomes may be caused by one or more factors. In our study, we hypothesized that TRAb levels are closely correlated with adverse outcomes of pregnancy. To demonstrate this correlation, we conducted a logistic regression analysis to assess the influencing factors of such outcomes (Table 3). The independent factors were age, body mass index, goiter, previous babies, previous miscarriage, previous preterm delivery, previous early preterm delivery, and TRAb. In this model, the reference group has no history of pregnancy, miscarriage and preterm delivery, goiter and negative TRAb. Additionally, the age and body mass index of the reference group were 20 years and 19.20 kg/m², respectively. The results show that the occurrence of pregnancy loss was independently associated with previous miscarriage

Table 3
Influencing factors of pregnancy loss^a.

Variable	<i>P</i> -Value ^b	Odds ratio	95% CI ^c
Previous miscarriage	0.03	2.20	1.08–4.50
TRAb	0.02	2.17	1.10–4.27

^a In the logistic regression model, the age and body mass index of the reference group were 20 years and 19.20 kg/m², respectively. Additionally, the reference group has no history of pregnancy, miscarriage and preterm delivery, goiter and negative TRAb.

^b Chi-square trend test was used to test the statistical difference in each group.

^c 95% Confidence interval.

(OR = 2.20, 95% CI: 1.08–4.50, $P < .05$) and TRAb (OR = 2.17, 95% CI: 1.10–4.27, $P < .05$). However, there was no significant correlation between these independent variables and preterm delivery ($P > .05$) and early preterm delivery ($P > .05$). TRAb was found to be an important influencing factor for pregnancy loss, making it useful for assessing the risk of pregnancy loss.

3.5. Comparison between TRAb and the combination of TSH, FT₄, and FT₃ for evaluating risk of pregnancy loss

The predictive value of TRAb in pregnancy loss was validated by comparing it with that of the combination of TSH, FT₄, and FT₃. The results are shown in Fig. 1. The optimal cutoff point for TRAb was 3.53 IU/L (Fig. 1A), and the sensitivity and specificity of TRAb to assess the risk of pregnancy loss are 83.5% and 85.3%, respectively (Fig. 1B). Meanwhile, the sensitivity and specificity of the combination was 70.3% and 84.6%, respectively (Fig. 1B). The ROC curves showed that TRAb has better sensitivity than the combination. The area under the ROC curve (AUC) was estimated according to the trapezoidal rule to be 95% confidence interval. The AUC was 0.86 (95% CI: 0.81–0.91) for TRAb and 0.83 (95% CI: 0.78–0.88) for the combination. These findings indicate that TRAb is more sensitive than the combination in the risk assessment of pregnancy loss in healthy pregnant women and is thus a more effective indicator.

4. Discussion

Thyroid dysfunction can considerably increase the risk of adverse pregnancy outcomes. In general, thyroid function during pregnancy is assessed according to the combination of TSH, FT₄, and FT₃ levels. Below normal TSH levels and above normal FT₄ and FT₃ levels indicate hyperthyroidism. Meanwhile, above normal TSH levels and below normal FT₄ and FT₃ levels indicate hypothyroidism [16]. Normal thyroid function does not altogether eliminate the risk of adverse outcomes as many pregnant women with normal thyroid function still develop adverse pregnancy outcomes due to the impact of thyroid autoantibodies.

The American Association of Clinical Endocrinologists stipulates that thyroid autoantibodies are an important indicator in the diagnosis of hyperthyroidism and hypothyroidism, and it is a factor that can be used to evaluate potential fetal risks in pregnant women with Graves' disease [17]. Therefore, the detection method for thyroid autoantibodies during pregnancy has been gradually developed in recent years [18–20]. Cotzias et al. detected thyroid autoantibodies during pregnancy and monitored the corresponding pregnancy outcomes; they concluded that thyroid autoantibodies may lead to the occurrence of adverse pregnancy outcomes [21]. Meanwhile, Matalon et al. investigated the effects of thyroid autoantibodies on pregnancy by determining thyroglobulin and thyroid peroxidase autoantibodies in pregnant women and also analyzed the association between thyroid autoantibody levels and miscarriages [22]. Their results showed that thyroid autoantibodies levels are correlated with pregnancy outcomes.

TRAb is an important autoantibody in ATD. However, TRAb has not

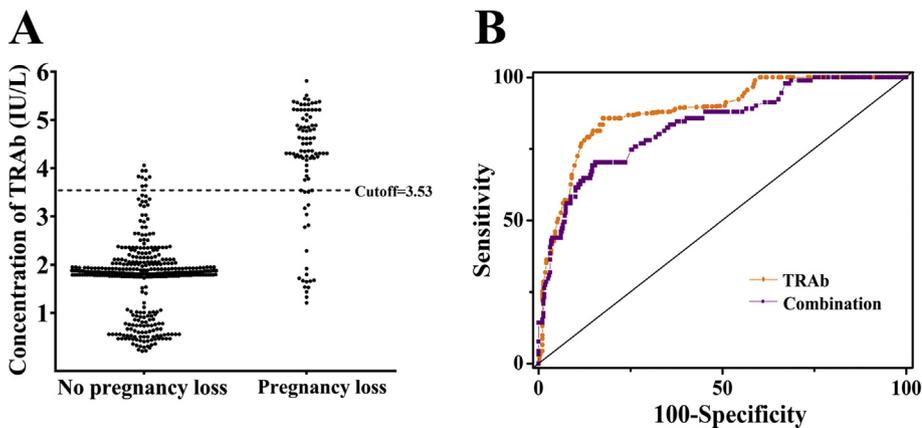


Fig. 1. Comparison of ROC curves between TRAb and the combination of TSH, FT₄, and FT₃ for evaluating risk of pregnancy loss. (A) Distribution of TRAb levels in women with ($n = 91$) and without ($n = 377$) pregnancy loss. TRAb levels ranged from 0.30 IU/L and 5.81 IU/L. (B) The sensitivity and the specificity of the combination was 70.3% and 84.6%, respectively. Meanwhile, the sensitivity and specificity of TRAb at its optimal cutoff point of 3.53 IU/L were 83.5% and 85.3%, respectively.

been considered as an effective risk assessment indicator for adverse pregnancy outcomes. In this study, we collected the data of 468 healthy pregnant women and evaluated the value of TRAb in the risk assessment of adverse pregnancy outcomes. The results show that TRAb level was significantly higher in women with pregnancy loss than that in women without pregnancy loss, and TRAb was an independent risk factor for pregnancy loss. Additionally, ROC curves showed that TRAb is a more sensitive indicator than thyroid function test in the risk assessment of pregnancy loss in healthy pregnant women. Unfortunately, TRAb was not an effective indicator for preterm delivery and early preterm delivery.

The Chinese government began to promote iodized salt in 1995 as part of a national policy to prevent iodine deficiency disorders (IDDs). Chongqing is an inland city in Southwest China, and it is affected by mild iodine insufficiency. Due to the lack of iodine-containing food in this area, the source of people's iodine intake is mostly derived from iodized salts [23]. However, with the rapid economic and transportation developments, other sources of iodine have become available to the residents. Thus, the incidence rate of IDDs in the population has been greatly reduced, and there is no longer any need to supplement iodine. However, the prevalence of thyroid disorders has also increased in recent years, reflecting the potential adverse effects of increased iodine intake [24]. Despite this, residents still commonly use iodized salt as the main edible salt because of old living habits.

Salt is one of the most important seasoning for flavoring food in China. Although the World Health Organization recommends a daily salt intake of 6 g, Chinese people generally consume > 20 g, particularly in Chongqing. The salt intake of residents in this region is higher than that in other parts of China due to particular dietary habits, and this may have led to the higher iodine intake in this region [25]. This may be the reason that the positive rate of TRAb is higher in Chongqing than that in other regions, but it needs to be verified by more population samples. Moreover, the incidence rate of thyroid disease is relatively higher in Chongqing than that in other regions [26]. Therefore, it was possible that hidden ATD patients in this study, which may have influenced the increased incidence rate of pregnancy loss in the results.

Although this study has some limitations, it provides important data for the risk assessment of adverse pregnancy outcomes. TRAb detection during pregnancy is a relatively new diagnostic tool in clinical practice in Southwest China, but it costs less than thyroid function test. Thyroid function is determined via laboratory evaluation of TSH, FT₄ and FT₃ levels and it costs approximately \$16. The TRAb assay is a separate test, and it costs only approximately \$ 8. Chongqing is an inland city in Southwest China, and the local economy is less developed than that in the eastern region because of certain limitations. As such, a less costly method for evaluating thyroid function would be beneficial and more acceptable to pregnant women in this area. In this regard, TRAb is extremely useful for improving the clinical management of thyroid function status in pregnant women.

5. Conclusions

In this study, we revealed that TRAb was associated with adverse pregnancy outcomes. The TRAb level considerably influenced pregnancy outcomes, and a high TRAb level may lead to adverse outcomes in pregnant women, particularly pregnancy loss. Moreover, TRAb was a more sensitive indicator to assess the potential risk of pregnancy loss than thyroid function test, and it can be used as a reliable factor to improve the clinical management of thyroid disease in pregnant women. This research provided a valuable indicator not only to measure the potential risk of adverse pregnancy outcomes, but also to assess the thyroid function status of pregnant women in clinical practice.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Southwest Hospital, Third Military Medical University, Chongqing, China. All participants provided written informed consent to allow their specimens to be used as part of the ongoing study on the TRAb assay and thyroid function test. A code used to replace the participant names, and any results were kept strictly confidential in protection of participant privacy rights.

Competing interests

The authors have no financial or non-financial interests in this study.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

Yuyan Li, Ting Xu, Qinyun Mo obtained informed consent from the participants, collected and analyzed the samples, and wrote the first draft of the manuscript. Weiling Fu and Chunyan Yao planned the study, performed the biometric calculations and revised the manuscript. All authors have read and approved the final version of the manuscript.

Acknowledgements

The authors are grateful to all of the clinical participants in this study for their support during sample collection and detection.

Declarations of interest

None.

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